(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 January 2004 (15.01.2004)

PCT

(10) International Publication Number
WO 2004/004642 A2

(51) International Patent Classification[7]: A61K

(21) International Application Number: PCT/US2003/020848

(22) International Filing Date: 2 July 2003 (02.07.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/393,932 3 July 2002 (03.07.2002) US

(71) Applicant (*for all designated States except US*): **THE UNIVERSITY OF TENNESSEE RESEARCH CORPORATION** [US/US]; 1534 White Avenue # 403, Knoxville, Tn 37996-1527 (US).

(72) Inventor; and
(75) Inventor/Applicant (*for US only*): **ZAGHOUANI, Habib** [TN/US]; 1608 Brookfield Manor, Columbia, MO 65203 (US).

(74) Agent: **WURST, John, E.**; Alliance Pharmaceutical Corp., 6175 Lusk Blvd, San Diego, CA 92121 (US).

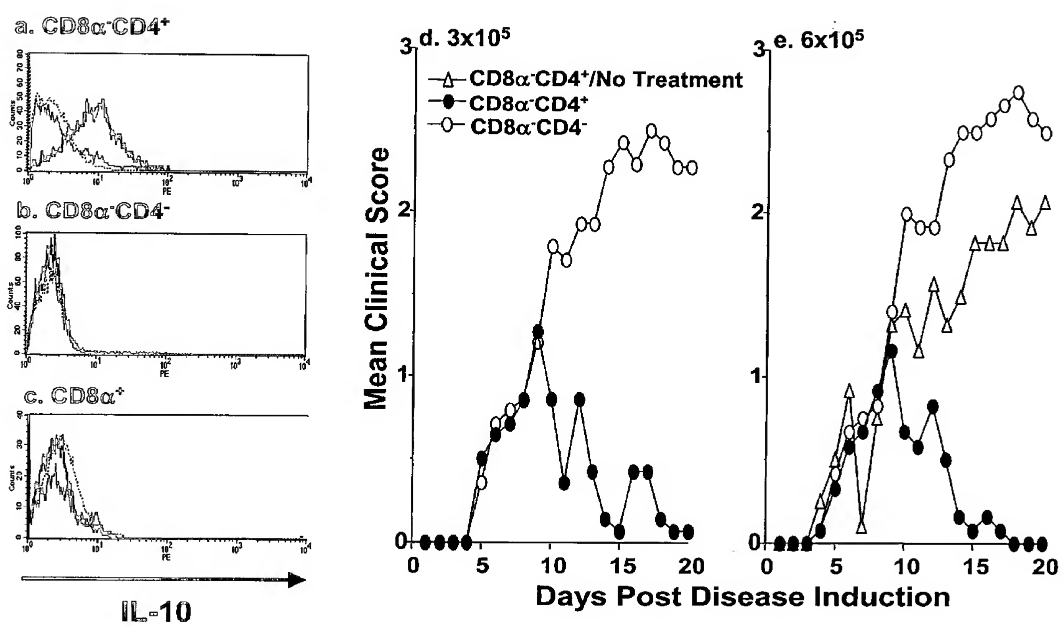
(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) **Title:** TCELL TOLERANCE AND MODULATION OF AUTOIMMUNITY BY CD8α⁻CD4⁺ DENDRITIC CELLS



(57) **Abstract:** The present invitation relates to compositions and methods for treatment of autoimmune disorders such as multiple sclerosis. More specifically, the present invention teaches the *in vivo* use of a subset of dendritic cells in conjunction with engineered immunoglobins containing a self-peptide which together have modulatory effects on tolerization of autoreactive T cells and sustain peripheral tolerance against self-antigens.

WO 2004/004642 A2

T Cell Tolerance and Modulation of Autoimmunity by CD8α^-CD4$^+$ Dendritic Cells

Cross Reference to Related Applications

The present application claims priority to U.S. Patent Application Serial No. 60/393,932 filed July 3, 2002, the contents of which are hereby incorporated by reference.

Field of the Invention

The present invention relates to compositions and methods for treatment of an autoimmune disorder. More specifically, the present invention relates to the use of a subset of dendritic cells in conjunction with engineered immunoglobulins for the treatment of autoimmune disorders including tolerization of autoreactive T cells and peripheral tolerance against self-antigens.

Background of the Invention

Dendritic cells ("DC" when singular and "DCs" when plural) represent a phenotypically heterogeneous population endowed with an important biologic function, namely, the presentation of antigens to both B and T lymphocytes (1-4)(see list of references at the end of the application). To date, it is believed that priming of naïve T cells and stimulation of primary T cell responses are mainly a function of DCs with minimal contribution from other professional antigen presenting cells ("APCs") (2, 3). Furthermore, recent evidence has indicated that different DC subsets confer discrete developmental functions to T cells (5 - 8). Accordingly, human DC1 and mouse CD8α^+ DC subsets were shown to promote differentiation into Th1 effectors, while human DC2 and mouse CD8α^- subsets supported development of Th2 cells (6-8). More recently, DCs have been shown to take up apoptotic bodies and necrotic debris (9, 10)

and cross-present (11) the exogenous antigens brought to the DCs by the dead cells by intersecting the endogenous pathway of antigen presentation. This phenomenon has proven quite potent in cross-priming cytolytic T lymphocytes (10-13). Moreover, this approach may prove valuable in therapeutic vaccination against tumors by circumventing the need for identification of the tumor-associated antigen and bypassing the tumor's poor immunogenicity (14 -15). The current knowledge on the roles that DCs play in T cell priming has been growing due to the availability of experimental models. However, little is known about the critical contribution of DCs to central tolerance (16 - 18) and overall knowledge on their role in peripheral tolerance is still in its infancy (19).

In prior studies, it was demonstrated that Ig-PLP1, an immunoglobulin ("Ig") chimera carrying the encephalitogenic PLP1 peptide corresponding to amino acid ("aa") sequence 139-151 of proteolipid protein ("PLP"), is presented to T cells ≈100 fold better than free PLP1 (20). In addition, aggregation of the chimera drives crosslinking of Fcγ receptors ("FcγRs") on APCs and endows Ig-PLP1 with an additional feature, namely, the induction of IL-10 by macrophages and DCs (26) without stimulating upregulation of costimulatory molecules (22). Consequently, mice induced for experimental allergic encephalomyelitis ("EAE") with central nervous system ("CNS") homogenate and treated with adjuvant free aggregated ("agg") Ig-PLP1 dramatically reduced their paralytic severity and promoted full recovery from EAE. A likely mechanism underlying the effective suppression of EAE by agg Ig-PLP1 maybe a synergy between efficient peptide presentation, lack of co-stimulation and IL-10-mediated bystander suppression (22).

In FcγR-deficient ("FcγR$^{-/-}$") mice, agg Ig-PLP1- mediated peptide presentation and IL-10 production by APC is compromised. Thus, these mutant mice offer a suitable host into which to transfer wild type DCs and evaluate their contribution to agg Ig-PLP1-mediated modulation of

autoreactive T cells and maintenance of peripheral tolerance. However, due to the lack of $Fc\gamma R^{-/-}$ mice on the SJL/J (H-2^s) background it was opted herein to instead engineer the I-A^b-restricted myelin oligodendrocyte glycoprotein ("MOG") aa 35-55 peptide (23) into the Ig backbone and use the resulting Ig-MOG chimera along with $Fc\gamma R^{-/-}$ C57Bl/6 mice (24-25) to evaluate the contribution of DCs to peripheral tolerance.

The following United States Patent Applications are hereby incorporated by reference in their entireties: U.S. Patent Application Serial Nos.: 08/779,767, entitled "Compounds, Compositions and Methods for the Endocytic Presentation of Immunosuppressive Factors", filed January 7, 1997; 10/277,264, entitled " Compounds, Compositions and Methods for the Endocytic Presentation of Immunosuppressive Factors" filed October 21, 2002; 09/111,123, entitled "Compounds, Compositions and Methods for the Endocytic Presentation of Immunosuppressive Factors", filed 6 July 1998; 09/623,728, entitled "Compounds, Compositions and Methods for the Endocytic Presentation of Immunosuppressive Factors", filed September 5, 2000; and, 09/873,901, entitled "Coupling of Peripheral Tolerance to Endogenous IL-10 Promotes Effective Modulation of T Cells and Ameliorates Autoimmune Disease", filed June 4, 2001.

Summary of the Invention

The present invention takes advantage of a well-characterized experimental allergic encephalomyelitis ("EAE") model of autoimmunity and evaluates the contribution of DCs to peripheral T cell tolerance and modulation of a paralytic autoimmune disease and autoimmune disorders in general. Herein, an APC transfer system is devised and applied to the autoimmune model of EAE, to evaluate the contribution that DCs play in peripheral T cell tolerance. The

CD8α⁻CD4⁺subset, a minor population among splenic DCs, is found to mediate both tolerance and bystander suppression against diverse T cell specificities. Aggregated ("agg") Ig-MOG, an Ig chimera carrying the myelin oligodendrocyte glycoprotein ("MOG") 35-55 peptide, binds and cross-links FcγR on APCs leading to efficient peptide presentation and IL-10 production. Furthermore, administration of agg Ig-MOG into diseased mice induces relief from clinical EAE involving multiple epitopes. Such recovery could not occur in FcγR-deficient mice where both uptake of Ig-MOG and IL-10 production are compromised. However, reconstitution of these mice with DC populations incorporating the $CD8\alpha^{-}CD4^{+}$ subset restored Ig-MOG-mediated reversal of EAE. Transfer of $CD8\alpha^{+}$ or even $CD8\alpha^{-}CD4^{-}$ DCs had no effect on the disease. These findings strongly implicate DCs in peripheral tolerance and emphasize their functional potency, as a small population of DCs was able to support effective suppression of autoimmunity.

The results obtained and presented in the present application demonstrate in part that agg Ig-MOG reverses clinical EAE induced in C57Bl/6 mice by injection of central nervous system ("CNS") homogenate, as did agg Ig-PLP1 in the SJL/J mice. This indicates that bystander suppression of diverse T cell specificities is also operative in the Ig-MOG/C57Bl/6 system. When a similar treatment regimen with agg Ig-MOG was applied to $Fc\gamma R^{-/-}$ C57Bl/6 mice, recovery from disease did not occur. This result was anticipated as both agg Ig-MOG-driven peptide presentation and IL-10 production by APCs would be compromised in these $Fc\gamma R^{-/-}$ mice. However, what was unexpected was that transfer of wild type C57Bl/6 DCs into the $Fc\gamma R^{-/-}$ mice prior to treatment with agg Ig-MOG restored Ig-MOG-mediated reversal of disease.

In order to gain further insight into how DCs operate the modulation of autoreactive T cells, DCs were separated into subsets and tested for both cytokine production *in vitro* upon

incubation with agg Ig chimeras and for restoration of agg Ig-chimera reversal of EAE in the $Fc\gamma R^{-/-}$ mice. The results indicate that upon cross-linking of FcγR by the Ig-chimeras, the $CD8\alpha^-$ DCs secreted IL-10 and reversed EAE in the mutant mice while the $CD8\alpha^+$ DCs were unable to reverse EAE and instead produced IL-12. More striking, further separation of the $CD8\alpha^-$ DCs into $CD8\alpha^-CD4^+$ and $CD8\alpha^-CD4^-$ populations indicated that IL-10 production was confined to the $CD8\alpha^-CD4^+$ subset, which also supported suppression of autoreactive T cells and reversal of EAE in the FcγR-deficient mice.

The present invention has application in the treatment of autoimmune diseases such as rheumatoid arthritis, insulin dependent diabetes, multiple sclerosis, lupus, scleroderma, and ulcerative colitis.

The following paragraphs represent nonlimiting embodiments of the invention:

1. A method of tolerizing autoreactive T cells in a subject suffering from an autoimmune disorder comprising administering dendritic cells to the subject in conjunction with a pharmaceutically effective amount of an aggregated immunoglobulin or portion thereof wherein the immunoglobulin or portion thereof is linked to a peptide or portion thereof wherein the peptide or portion thereof is associated with the autoimmune disorder.

2. The method of paragraph 1 wherein the dendritic cells are administered to the subject by adoptive transfer.

3. The method of paragraph 2 wherein the dendritic cells are administered in combination with a pharmaceutically acceptable carrier.

4. The method of paragraphs 1 - 3 wherein the dendritic cells are administered together with or in close proximity with aggregated immunoglobulin-peptide composition (Ig-chimera).

5. The method of paragraph 1 wherein the autoimmune disorder is multiple sclerosis and the T cells are myelin specific T cells.

6. The method of paragraphs 1 - 5 wherein the tolerization of autoreactive T cell includes tolerization and suppression of T cells.

7. The method of paragraphs 1 - 5 wherein the DCs are CD8α- dendritic cells.

8. The method of paragraphs 1 - 4 wherein the autoimmune disorder is multiple sclerosis.

9. The method of paragraph 8 wherein the subject is administered in conjunction with the dendritic cells a composition from the group consisting of agg Ig-MOG and agg Ig-PLP1.

10. The method of paragraphs 2, 7 and 8 wherein the method results in enhanced IL-10 production and decreased IL-12 production.

11. The method of paragraphs 2, 7, 8 and 9 wherein the method results in increased bystander regulation and increased peripheral tolerance of the autoimmune disorder.

12. The method of paragraph 2 wherein the dendritic cells are loaded with antigenic peptide prior to adoptive transfer of the dendritic cells into the subject.

13. The method of paragraph 7 wherein the CD8α- dendritic cells are CD8α-CD4$^+$ cells.

14. A method of treating an immune disorder comprising administering to a patient by adoptive transfer a therapeutically effective amount of dendritic cells in conjunction with a peptide associated with an autoimmune disorder.

15. The method of paragraph 14 wherein the immune disorder is multiple sclerosis wherein the peptide is administered with an immunoglobulin.

16. The method of paragraphs 14 - 15 wherein the dendritic cells are CD8α- dendritic cells.

17. The method of paragraphs 14 - 16 wherein the dendritic cells are CD8α-CD4$^+$ dendritic cells.

18. The method of paragraph 17 wherein the production of IL-10 is significantly enhanced.

19. The method of paragraph 17 wherein the production of IL-12 is significantly decreased.

20. The method of paragraphs 14 wherein the dendritic cells are loaded with peptide prior to adoptive transfer.

21. The method of paragraphs 16 - 17 wherein the administration of dendritic cells tolerize T cells which are myelin specific T cells.

22. The method of paragraph 14 wherein the dendritic cells tolerize pathogenic Th1 cells and enhance IL-10 production.

23. The method of paragraphs 14 - 15 wherein the dendritic cells are administered to the patient adjuvant free.

24. A method of treating or preventing multiple sclerosis in a subject in need thereof comprising: administering to the subject a pharmaceutically effective amount of dendritic cells which may optionally be administered together with a pharmaceutically effective amount of a composition comprising an immunoglobulin or portion thereof, linked to a peptide, wherein the peptide is derived from a self-antigen associated with multiple sclerosis.

25. The method of paragraph 24 wherein the immunoglobulin or portion thereof is agg Ig-PLP1 or agg Ig-MOG.

26. The method of paragraph 24 wherein the agg Ig-PLP1 or agg Ig-MOG crosslink the FcγR1 receptors on the dendritic cells.

27. The method of paragraph 24 wherein the immunoglobulin is human IgG or humanized IgG.

28. The method of paragraph 24 wherein the immunoglobulin or portion thereof is aggregated.

29. The method of paragraph 24 wherein the PLP1 or MOG are incorporated into human or humanized IgG and Ig-PLP1 or Ig-MOG is aggregated.

30. The method of paragraph 24 wherein the subject is in the preclinical stage of multiple sclerosis.

31. The method of paragraph 24 wherein the dendritic cells are administered by adoptive transfer.

32. The method of paragraph 24 wherein the dendritic cells are administered intraveneously.

33. The method of paragraph 24 wherein the dendritic cells are CD8α- dendritic cells.

34. The method of paragraph 24 wherein the dendritic cells are CD8α-CD4$^+$ dendritic cells and induce production of T reg cells.

35. The method of paragraphs 24 and 32 -33 wherein the dendritic cells are CD8α-CD4$^+$ dendritic cells and enhances bystander regulation.

36. The method of paragraph 35 wherein administration of the composition reverses the course of multiple sclerosis.

37. The method of paragraph 24 wherein the administration of the composition induces production of IL-10 thereby enhancing bystander suppression of pathogenic T cells.

38. The method of paragraph 24 wherein the method tolerizes autoreactive T cells.

39. The method of paragraph 24 wherein the composition tolerizes autoreactive Th1 cells.

40. The method of paragraph 38 wherein the T cells are specific for myelin proteins.

41. The method of paragraph 24 wherein the dendritic cells and composition may be administered in a pharmaceutically acceptable carrier.

42. Use of a composition for the treatment of an autoimmune disorder wherein the composition comprises a pharmaceutically effective amount of dendritic cells wherein the dendritic cells are administered to a patient suffering from the autoimmune disorder in conjunction with a pharmaceutically effective amount of an immunoglobulin or portion thereof , linked to one or more peptides derived from self antigens.

43. The use of paragraph 42 wherein the immunoglobulins are aggregated.

44. The use of paragraph 42 wherein the immunoglobulin or portion thereof binds to the Fc receptor of the dendritic cells and is endocytosed by the dendritic cells and the one or more

peptides, or portion thereof, is processed and presented by the dendritic cells in association with MHC Class II molecules thereby tolerizing autoreactive T cells and thereby alleviating symptoms of the autoimmune disorder.

45. The use of paragraph 42 wherein the autoimmune disorder is multiple sclerosis.

46. The use of paragraph 42 wherein the immunoglobulin or portion thereof is selected from the group consisting of Ig-MOG and Ig-PLP1.

47. The use of paragraph 45 wherein the peptide is derived from proteolipid protein.

48. The use of paragraph 42 wherein the autoimmune disorder is selected from the group consisting of type 1 diabetes, multiple sclerosis and rheumatoid arthritis.

49. The use of paragraph 42 wherein the autoimmune disorder is a T cell mediated autoimmune disorder.

50. The use of paragraph 42 wherein the peptide is linked within a variable region of the immunoglobulin.

51. The use of paragraphs 42 - 44 wherein the immunoglobulin or portion thereof is humanized immunoglobulin.

52. The use of paragraphs 42 - 51 wherein IL-10 production is enhanced and IL-12 production is decreased thereby enhancing bystander suppression against the autoimmune disorder.

53. The use of paragraphs 42 - 52 in the manufacture of a medicament for treatment or prevention of an autoimmune disorder.

54. A method of tolerizing autoreactive T cells in a subject suffering from an autoimmune disorder comprising loading of dendritic cells *in vitro* with a peptide associated with the autoimmune disorder and administering the dendritic cells by adoptive transfer to the subject.

55. The method of paragraph 54 wherein the autoimmune disorder is a T cell mediated autoimmune disorder.

56. The method of paragraph 54 wherein the autoimmune disorder is multiple sclerosis and the peptide is selected from the group consisting PLP1 and MOG.

57. The method of paragraphs 54 - 56 wherein the method tolerizes and suppresses autoreactive T cells.

58. The method of paragraph 57 wherein the T cells are myelin specific T cells.

59. The method of paragraph 54 wherein the dendritic cells are CD8α-CD4^{+} dendritic cells.

60. The method paragraphs 54 - 59 wherein the adoptive transfer of the dendritic cells results in enhanced IL-10 production and decreased IL-12 production.

61. The method paragraphs 54 - 59 wherein the method results in increased bystander regulation and increased peripheral tolerance of the autoimmune disorder.

Brief Description of the Drawings:

Figure 1, panels (a) and (b), show that agg Ig-MOG modulates disease involving diverse T cell specificities;

Figure 2, panels a - d, show that the induction of IL-10 production by agg Ig-chimeras is mediated primarily by FcγR I;

Figure 3 shows that DCs mediate reversal of EAE by agg Ig-chimeras;

Figure 4 shows that DCs produce IL-10 and down-regulate IL-12 upon incubation with agg Ig chimeras;

Figure 5, panels a - d, show that CD8α$^-$ DCs produce IL-10 while CD8α$^+$ secrete IL-12 in response to agg Ig-chimeras;

Figure 6, panels a - f, show that CD8α$^-$ DCs are unable to promote the production of IFNγ by T cells;

Figure 7, panels a and b, CD8α$^-$ wildtype DCs restore agg Ig-MOG mediated reversal of EAE in FcγR I, III$^{-/-}$ mice;

Figure 8 demonstrates that CD8α$^+$, but not CD8α$^-$, DCs promote the development of EAE; and,

Figure 9, panels a - d, show that CD8α$^-$CD4$^+$ DCs produce IL-10 in response to agg Ig-chimeras.

Detailed Description of the Invention

A) Materials and Methods

Animals

SJL/J mice were purchased from Harlan-Sprague-Dawley (Frederick, MD) and C57Bl/6 mice from The Jackson Laboratory (Bar Harbor, ME). Fcergl (FcγR I, $III^{-/-}$), Fcgr2b (FcγR II $^{-/-}$) and Fcer1g/Fcgr2 (FcγR I, II, III $^{-/-}$) mice (24, 25) were purchased from Taconic (Germantown, NY). All mice were bred and maintained in an animal care facility for the duration of the experiments. All experimental procedures were carried out according to the guidelines of the institutional animal care committee.

Antigens

Peptides: The peptides used in this study were purchased from Research Genetics (Huntsville, AL) and were HPLC purified to >90% purity. PLP1 peptide [Seq. I.D. No. 1 (HSLGKWLGHPDKF)] encompasses aa residues 139-151 of PLP and is encephalitogenic in SJL/J mice (26). MOG peptide [Seq. I.D. No. 2 (MEVGWYRSPFSRVVHLYRNGK)], encompassing aa residues 35-55 of MOG, is encephalitogenic in C57Bl/6 mice (23).

CNS homogenate: Fifty frozen unstripped rat brains (Pelfreez Biologicals, Rodgers, AK) were homogenized in PBS using a Waring blender and adjusted to 300 mg/ml with PBS. CNS homogenate was stored at –20°C.

Ig-chimeras: Ig chimeras. Ig-PLP1 and Ig-MOG were constructed to express the PLP1 and MOG peptides as taught in the Examples. The Ig-PLP1 chimera harbors PLP1 peptide within the heavy chain CDR3 region and has been previously described (20 - 22), the contents of which are hereby incorporated by reference. The Ig-MOG chimera harbors MOG 35-55 peptide

within the heavy chain CDR3 region and, like Ig-PLP1, was constructed using the genes coding for the BALBC IgG2b per the teachings of Gilian et. al., *Cell.* 33:717 (1983), κ anti-arsonate antibody, 91A3 as described in Gary et al., *Proc. Natl. Acad. Sci.,* 84:1085 - 1089, Ruthban et al., *J. Mol. Biol.* 202:383 - 395 (1988) and (20), all of which are incorporated by reference. Deletion of the heavy chain CDR3 region and replacement with nucleotide sequences coding for PLP1 and MOG are similar to those described for the generation of Ig-NP (32) and Ig-HA (33), the contents of which are hereby incorporated by reference.

Briefly, the D segment was deleted from the CDR3 of the 91A3 heavy chain variable region and replaced with a nucleotide sequence [Seq. I.D. No. 3 (ADGGAGGTCGGATGGTACAGGAGTCCCTTTTCTCGAGTTGTCCACCTCTATAGGAA CGGAAAG)] that code for MOG35-55 peptide using mutagenesis procedures similar to those described for the generation of Ig-PLP1 (20), Zaghouani et al., *J. Immunol.* 185:1043 - 1053 (1997) and Ig-NP, Zaghouani et al., *J. Immunol.*, 148: 3604 - 3609 (1992), all of which are hereby incorporated by reference. These references report that the CDR3 region of the 91A3 IgG is compatible for peptide expression and that both class I and class II restricted epitopes have been efficiently processed and presented to T cells when grafted in place of the naturally occurring segment.

Briefly, the $91A3V_H$ gene was subcloned into the EcoR1 site of pUC19 plasmid and used as a template DNA in PCR mutagenesis reactions to generate $91A3V_H$ fragments carrying MOG ($91A3V_H$ - MOG) and PLP1 ($91A3V_H$ - PLP1) sequences in place of CDR3. Nucleotide sequencing analysis indicated that the full MOG and PLP1 sequences were inserted in the correct reading frame (not shown). The $91A3V_H$ - MOG and $91A3V_H$ - PLP1 fragments were then subcloned into the EcoR1 site of pSV2-gpt-$C\gamma 2b$ in front of the exons coding for the constant

region of BALB/c γ2b that that generated pSV2-gpt-91A3V_H-MOG-Cγ2b and pSV2-gpt-91A3V_H-PLP1-Cγ2b plasmids, respectively. These plasmids were then separately co-transfected into the non-Ig producing SP2/0 myeloma B cell line with an expression vector carrying the parental 91A3 light chain, pSV2-neo-91A3L. Transfectants producing Ig chimeras were selected in the presence of geneticin and mycophenolic acid. Transfectants were cloned by limiting dilution and final clones secreted 1 - 4 μg/ml of Ig-MOG or Ig-PLP1 chimeras. Conventional transfection, cloning, sequencing, and purification procedures for Ig-MOG are similar to those used for Ig-PLP1 a taught in (20) and Zaghouani et al., *J. Immunol.* 148:3604 - 3609, both of which are hereby incorporated by reference. The cell lines and Ig-chimeras are maintained in permanent storage in the inventor's laboratory.

Large-scale cultures of Ig-MOG and Ig-PLP1 transfectants were performed in DMEM containing 10% serum supreme (BioWhittaker, Walkersville, MD) and purified on separate rat anti-mouse κ chain sepharose columns to avoid cross-contamination. Subsequently, the Ig-chimeras were dialyzed against PBS and concentrated on collodion membranes (Schleicher & Schuall, Keene, NH). The chimeras were aggregated by precipitation with 50%-saturated $(NH_4)_2SO_4$ as has been previously described (21, 22), the contents of which are hereby incorporated by reference. Since both Ig-PLP1 and Ig-MOG derive from the same Ig backbone and thereby comprise identical IgG2b isotype, their Fc associated functions including binding and cross-linking of FcγR will be similar. In this respect we may refer to them indistinguishably as Ig-chimeras.

It is also within the scope of the invention herein that the immunoglobulin or portion thereof has more than one peptide linked to the immunoglobulin or portion thereof. Furthermore, the immunoglobulin, or portion thereof, can be human or humanized, such as for example,

human IgG, such as IgG1, IgG2, IgG2, IgG3 and IgG4. The Ig chimeras of the present invention may also comprise and be administered with an acceptable pharmaceutical carrier.

It is also within the scope of the invention to use free peptides or peptides in an acceptable pharmaceutical carrier to load dendritic cells *in vitro* prior to adoptive transfer. For example, it would be possible to load dendritic cells with any of the peptides mentioned herein or other peptides associated with autoimmune disorders and administer the dendritic cells to the host.

Adoptive transfer of the dendritic cells involve well known steps in the art including purifying the dendritic cells, isolating the subset (see "Isolation of Dendritic Cells") and injecting the dendritic cells into the host intravenously or by other modes of administration. By "host" or "subject" it is intended to include both human and non-human "hosts" or "subjects" and in humans it is intended to include embryonic, fetal, neonatal, infant, juvenile and adults.

Isolation of Dendritic cells

Dendritic cells: Splenic DCs were purified according to the standard collagenase/differential adherence method (27), the contents of which are incorporated by reference. Briefly, the spleen was disrupted in a collagenase solution, and isolated DCs floated on a dense BSA gradient. Subsequently, the cells were allowed to adhere to petri dishes for 90 minutes at 37°C, washed, and incubated overnight. For isolation of DC subsets, bulk DCs were incubated with anti-CD8α mAb coupled microbeads (Miltenyi Biotec, Auburn, CA) and separated into CD8α^+ and CD8α^- populations by magnetic activated cell sorting (MACS®) (Miltenyi Biotec). The CD8α^- fraction was repassed on magnetic columns following incubation with anti-CD8α^+ mAb coupled microbeads to eliminate any residual CD8α^+ cells. Subsequently,

the $CD8\alpha^-$ cells were further purified by positive selection using anti-CD11c mAb coupled microbeads (Miltenyi Biotec).

For preparation of $CD8\alpha^-CD4^+$ and $CD8\alpha^-CD4^-$ DC subsets, the $CD8\alpha^-$ fraction was labeled with anti-CD4 mAb coupled microbeads (Miltenyi Biotec), and the subsets were separated as above. The $CD4^-$ fraction was further purified by positive selection using anti-CD11c mAb coupled microbeads (Miltenyi Biotec). Each fraction was assessed for purity and no population was used if contamination was greater than 5%.

Induction of EAE

6-8 wk old mice were induced for EAE by subcutaneous injection in the footpads and at the base of the limbs and tail with a 200 μl IFA/PBS (v/v) solution containing 6 mg CNS homogenate and 200 μg *Mycobacterium tuberculosis* H37Ra (Difco Laboratories, Detroit, MI). Six hours later the mice were given intravenously 200 ng of purified *Bordetella pertussis* toxin (List Biological Laboratories, Inc., Campbell, CA). A second injection of *B. pertussis* toxin was given after 48 hours. Subsequently, the mice were scored daily for clinical signs of EAE as follows: 0, no clinical score; 1, loss of tail tone; 2, hindlimb weakness; 3, hindlimb paralysis; 4, forelimb paralysis; and 5, moribund or death. EAE induced by CNS homogenate manifests as a monophasic disease in C57Bl/6 mice and as a relapsing/remitting disease in SJL/J mice. For induction of EAE with peptide pulsed DCs, the following procedure was used: purified $CD8\alpha^+$ or $CD8\alpha^-$ DCs (1x 10^6/ml) were pulsed overnight with 50 μg/ml PLP1 peptide, washed, resuspended in PBS, and injected subcutaneously into the hindlimb footpads of mice at 3 x 10^5 cells/mouse. Six and twenty four hours later, the mice were given i.v. 200 ng of *B. pertussis* toxin.

Treatment of EAE

Treatment with Peptide: Mice induced for EAE with CNS homogenate began receiving treatment with 100 μg of PLP1 or MOG peptide after loss of tail tone, which occurs regularly between days 6 and 8 post disease induction. Treatment injections were given intraperitoneally in PBS on days 9, 13 and 17 as previously described (21).

Treatment with Ig-PLP1 or Ig-MOG: Mice induced for EAE with CNS homogenate began receiving treatment with 300 μg of agg Ig-PLP1 or Ig-MOG after loss of tail tone. Treatment injections were given intraperitoneally in PBS on days 9, 13 and 17 after disease induction as previously described (21). Treatment of EAE in $Fc\gamma R^{-/-}$ mice reconstituted with wildtype APCs was carried out as follows: on day 5 following disease induction, the mice were adoptively transferred intraperitoneally with either 0.3 or 0.6 x 10^6 purified APCs. Subsequently, the animals were treated with agg Ig-MOG on days 9, 13 and 17.

Cytokine detection

ELISA: ELISA was performed according to BD PharMingen's standard protocol. The capture Abs were as follows: rat anti-mouse IFNγ, R4-6A2; rat anti-mouse IL-10, JES5-2A5; rat anti-mouse IL-4, 11B11; and, rat anti-mouse IL-12, 9A5. The biotinylated anti-cytokine Abs were as follows: rat anti-mouse IFNγ, XMG1.2; rat anti-mouse IL-10, JES5-16E3; rat anti-mouse IL-4, BVD6-24G2; and, rat anti-mouse IL-12, C17.8. All antibodies were purchased from BD Pharmingen, San Diego, CA. Assays were read on a SPECTRAmax 190 counter (Molecular Devices, Sunnyvale, CA). Graded amounts of recombinant mouse IL-4, IFNγ (BD Pharmingen), IL-10, or IL-12 (Peprotech, Rocky Hill, NJ) were included in all experiments for construction of

standard curves. The cytokine concentration in culture supernatants was estimated by extrapolation from the linear portion of the standard curve.

Intracellular cytokine staining: CD8α^+, CD8α^-CD4$^+$, or CD8α^-CD4$^-$ DCs (1x10^6/ml/well) were incubated with 0.6 μM agg or soluble (sol) Ig-chimera for 24 hours to allow for FcγR crosslinking. During the last 8 h of incubation, brefeldin A (10 μg) (Epicentre Technologies, Madison, WI) was added to the wells in order to block cytokine secretion. The cells were then harvested and stained with anti-CD11c-PerCP mAb, HL3, and FITC labeled anti-CD8α mAb, 53-6.7, or anti-CD4 mAb, GK1.5. Subsequently, the cells were fixed, permeabilized with 0.5% saponin (Sigma, St. Louis, MO), and stained for intracellular IL-10 using PE-labeled anti-IL-10 mAb, JES5-16E3. All antibodies used for staining were purchased from BD PharMingen, San Diego, CA. The FACS® data were analyzed on a FACScan Flow Cytometer (Becton Dickinson, Mount View, CA) using CellQuest software (Becton Dinkinson, Mount View, CA).

Stimulation of cytokine production by APCs

Splenocytes and subsets of DCs from normal or FcγR-deficient mice were plated with graded amounts of sol or agg Ig-PLP1, and the culture was then incubated for 24 h. Detection and quantification of cytokines was then assessed by ELISA from 100 μl of culture supernatant as described above.

T Cells

TCC-PLP1-1B10: The generation of the PLP1-specific Th0 cell clone, TCC-PLP1-1B10, has been previously described (21), the contents of which are hereby incorporated by reference. Briefly, SJL mice were immunized with 100 μg PLP1 peptide in CFA, and, 10 days later, the

draining lymph nodes were removed and stimulated with PLP1. After three stimulation/resting cycles, the cells were cloned twice by limiting dilution, and positive clones were retested for reactivity with PLP1 peptide by both proliferation and cytokine production. Subsequently, one positive clone displaying a Th0 pattern was selected and designated TCC-PLP1-1B10.

MOG-specific T cell line: A similar procedure was used to generate a MOG reactive T cell line in C57Bl/6 mice, and these cells were used for testing Ig-MOG for peptide presentation.

Analysis of cytokine responses upon presentation of agg Ig-PLP1 by subsets of DCs

Purified bulk, CD8α^+, or CD8α^- DCs were plated at 5 x 10^4 cells/well/50 μl and incubated with graded amounts of sol or agg Ig-PLP1 (100 μl/well) for 1 h. Subsequently, TCC-PLP1 1B10 cells (5 x 10^4 cells/well/50μl) were added, and the culture was continued for 24 h. Detection and quantification of cytokines were assessed by ELISA from 100 μl of culture supernatant as previously described.

B) Results

The present application demonstrates in part that C57Bl/6 mice recover from EAE when treated with agg Ig-MOG as did SJL/J mice following treatment with agg Ig-PLP1 (Figure 1). However, C57Bl/6 mice deficient for FcγR I and III could not resolve EAE upon similar treatment with agg Ig-MOG unless reconstituted with DCs from wild type C57Bl/6 mice prior to the treatment (Figure 3).

Previously, it has been shown that APC internalize Ig-chimera of the same IgG2b backbone as Ig-MOG and Ig-PLP1 mainly via FcγRs leading to processing of the chimeras within endosomes and efficient loading of the incorporated peptide onto newly synthesized MHC

molecules (40). Aggregation of the Ig chimeras adds another feature to the Ig delivery system, namely the cross-linking of FcγR and production of IL-10 by the APC (21, 22). FcγRI was identified as the main target for Ig-MOG and mice lacking such a receptor could not resolve EAE (Figure 3) most likely due to compromised presentation of Ig-MOG and lack of IL-10 production (Figure 2). Furthermore, the present application demonstrates that DCs produce IL-12 upon cross-linking of their FcγR but that the simultaneous production of IL-10 negatively regulates such IL-12 secretion (Figure 4). This observation prompted the question whether the two cytokines were produced by the same or by separate subsets of DCs and whether there was a correlation between a specific population of DCs and recovery from disease. The findings indicate that the IL-10 production is mediated by $CD8\alpha^-$ DCs and IL-12 by the $CD8\alpha^+$ population (Figure 5). This result is also supported by the observation that $CD8\alpha^-$, but not $CD8\alpha^+$ DCs, mediate recovery from EAE in the FcγR I, $III^{-/-}$ mice upon treatment with Ig-MOG (Figure 7). Although different subsets of DCs may display different pattern of trafficking (38) and migration issues could be at play in this form of peripheral tolerance, a number of observations argue instead that these results represent intrinsic functions of the two populations.

Figure 6 shows that upon incubation with agg Ig-PLP1, the $CD8\alpha^+$ DCs produce IL-12, present the PLP1 peptide, and stimulate the PLP1-specific Th0 cells to produce IFNγ. Therefore, these cells most likely would not support reversal of EAE upon encounter with pathogenic T cells when properly located. However, $CD8\alpha^-$ DCs produced IL-10 did not stimulate IFNγ production by the same Th0 cells (Figure 6) and therefore supported the reversal of EAE (Figure 7). Thus, in this *in vitro* T cell regulation system, where migration issues should not be at play, the DC subsets displayed functions that parallel the results obtained *in vivo*. Furthermore, when the two subpopulations were loaded with free peptide *in vitro* and then used to induce EAE, only

the $CD8\alpha^+$ DCs produced paralysis. This observation suggests that both naïve self-reactive T cells were available and that $CD8\alpha^+$ DCs were able to encounter these cells and drive their activation and induction of paralysis. However, the $CD8\alpha^-$ DCs population seems to mediate unresponsiveness as they did not activate the T cells to produce EAE despite their ability to migrate through the circulation, encounter the T cells and mediate reversal of EAE as shown in Figure 7. Thus, the intrinsic function of these cells most likely dictates the type of contribution that a DCs subset displays in this model of peripheral tolerance. However, it cannot be ruled out that differential trafficking patterns between the two populations influence their functions.

In recent years, reports have been made indicating that human immature DCs cross-presenting viral (42) or allogeneic (43) antigens induce IL-10-producing regulatory T cells that could potentially sustain peripheral tolerance. In addition, immature DCs were found to internalize apoptotic bodies generated from normal cells dying as a consequence of physiologic turnover, and transport the associated self-antigens to nearby lymph nodes populated with T cells (10, 44). Since T cell responses to those self-antigens were undetectable it was presumed that such transport served to maintain peripheral T cell tolerance (19, 44). The contribution of DCs to peripheral tolerance, however, remains largely undefined. More recently, it has been shown that repetitive injections of $TNF\alpha$-matured peptide-loaded DCs can protect against EAE presumably via induction of IL-10-producing regulatory T cells (45). Moreover, peptide loading into the endosomal compartment of DCs in the absence of inflammation also supports T cell down-regulation and likely maintains peripheral tolerance (46). The Ig chimeras drive both peptide loading into endosomes without causing inflammation and induce IL-10 production by APC aligning powerful factors against the self-reactive T cells. Interestingly, among the mature splenic DCs, the $CD8\alpha^-$ $CD4^+$ subset seems to drive such tolerance as they produce IL-10 and

support reversal of EAE in the FcγR I, III $^{-/-}$ mice (Figure 9). Given the small size of the CD8α$^-$ CD4$^+$ DCs population, it is suspected that such potency emanated from coordinated inhibitory functions. IL-10 produced by the CD8α$^-$ CD4$^+$ DCs could inhibit the function of T cells (47) directly by alteration of CD28 expression (33) and/or indirectly by down-regulation of IL-12 from the CD8α$^+$ DCs (Figure 4 and 6). Furthermore, it is believed that the CD8α$^-$ DCs and more specifically the CD8α$^-$CD4$^+$ subpopulation display tolerizing effects on Th1 cells as they were unable to drive IFNγ production by the Th0 T cell clone even upon stimulation with sol Ig-PLP1, a chimera which does not induce IL-10 production (Figure 6). The observation that CD8α$^-$ DCs loaded with free PLP1 peptide (hence no IL-10 involvement) were unable to induce EAE, while CD8α$^+$ DCs did (Figure 8), further supports the idea that CD8α$^-$ DCs are toleragenic against Th1 cells.

CD8α$^-$ DCs, which proved toleragenic against Th1 responses in this model, have previously been shown to promote the development of Th2 cells (7, 8). Thus, the question that arises is whether the same DCs display a functional plasticity and prime Th2 responses while tolerizing for Th1 immunity or if each function is confined to a distinct subset within the CD8α$^-$ population. The latter was confirmed for the tolerizing function as the CD8α$^-$CD4$^+$, but not the CD8α$^-$CD4$^-$ subset, was found to produce IL-10 and reverse EAE upon treatment with agg Ig-MOG (Figure 9). Therefore, it is believed that the CD8α$^-$CD4$^+$ subset of mature splenic DCs displays intrinsic tolerizing properties complemented with the ability to produce IL-10 and play a critical role in peripheral tolerance against self-reactive T cells. Whether or not these cells represent the previously described (6, 19, 48) mature DCs subset specialized in T cell tolerance is unclear.

Prior studies of disease reversal using agg Ig-PLP1 in the SJL system have not been able to detect regulatory T cells (21). Since regulatory T cells usually adopt a cycling pattern (49) and may be subject to self-limitation upon modulation of the pathogenic T cells, they may be difficult to detect subsequent to recovery from disease. If this is the case, the possibility remains that $CD8\alpha^{-}CD4^{+}$ DCs are able to induce regulatory T cells, particularly that they produce IL-10, a growth factor for regulatory T cells (34). Whatever the mechanism might be, these cells seem to be very potent, as tolerance has occurred in two different antigenic systems using two different mouse strains and the disease was reversed even in SJL mice where the autoreactive T cell repertoire encompasses an usual high frequency of self-reactive T cells (50).

Overall, these investigations have identified a DC subset that displays powerful modulatory effects on autoreactive T cells and provide a DC candidate that could sustain peripheral tolerance against self-antigens and suppress autoimmune disorders such as rheumatoid arthritis, insulin dependent diabetes, multiple sclerosis, lupus, scleroderma, and ulcerative colitis.

1) Examples:

It was previously demonstrated that agg Ig-PLP1 is effective in the induction of IL-10 by APC and modulation of EAE involving diverse T cell specificities (21, 22). Furthermore, the present application indicates that IL-10-mediated bystander suppression plays a critical role in T cell modulation and amelioration of EAE, as administration of anti-IL-10 mAb into mice during treatment of disease with agg Ig-PLP1 restored full severity of paralysis (21, 22). This experimental model of T cell tolerance was utilized in conjunction with FcγR-deficient mice to devise an adoptive APC transfer system suitable for evaluation of the role of DCs in peripheral tolerance.

Example 1: Broad efficacy of aggregated Ig-myelin chimeras in the suppression of EAE.

Since FcγR deficient mice were not available in the $H-2^s$ (SJL/J) background, C57Bl/6 ($H-2^b$) mice were chosen and an Ig-MOG chimera was engineered containing the $I-A^b$ restricted MOG35-55 peptide (see "Materials and Methods"), an epitope that has been shown to induce EAE in C57Bl/6 mice (23). To insure that the MOG peptide would indeed be processed from the chimera and presented to specific T cells, Ig-MOG was assayed for stimulation of a T cell line generated against MOG35-55 peptide. The results demonstrated that the MOG-specific T cell line significantly proliferated when it was incubated with Ig-MOG in the presence of compatible APC, thus indicating that Ig-MOG was taken up and processed by the APC and that a MOG peptide was generated and presented to the T cells (not shown in figures).

Subsequently, Ig-MOG was tested for amelioration of EAE in C57Bl/6 mice side-by-side with Ig-PLP1 in the SJL strain. Groups of SJL/J (a) or C57Bl/6 (b) mice were induced for EAE with 6 mg of CNS homogenate and were treated intraperitoneally with a saline solution containing 300 μg of agg Ig-PLP1, 300 μg agg Ig-MOG, 100 μg of free PLP1 peptide, or 100 μg MOG peptide (MOG peptide) on days 9, 13 and 17 post disease induction. On the basis of the molecular weight of the Ig-chimeras and PLP1 and MOG peptide, it was estimated that 300 μg of free peptide contains 17 and 19 fold higher copies of peptide, respectively, than the Ig-chimera. Groups of untreated mice (Nil) were included for comparison purposes. The clinical onset of disease was at day 5-7 post disease induction in these experimental groups. Each point represents the mean clinical score of 6 mice.

The results, as indicated in Figure 1, panels a and b, indicate that agg Ig-MOG, like agg Ig-PLP1, was able to reverse CNS homogenate induced EAE and, most likely, involved down-

regulation of diverse T cell specificities. Indeed, while the untreated C57Bl/6 mice had a mean maximal score of 2.4 ± 0.2, those treated with agg Ig-MOG had a mean maximal score of 1.2 ± 0.3 (Figure 1, panel b). In addition, while agg Ig-MOG treated animals fully recovered from the typical monophasic EAE by day 22 post disease induction, the recovery of the untreated mice was delayed and occurred on day 47. This concurs with the SJL/Ig-PLP1 system in which the untreated mice had a mean maximal disease score of 2.8 ± 0.3 and had not recovered by day 60 post disease induction, as relapses were still observed. Those treated with agg Ig-PLP1 had a mean maximal disease score of 1.4 ± 0.3 and fully recovered from this relapsing/remitting disease by day 26 post induction, and relapses were not observed (Figure 1, panel a). Furthermore, free PLP1 and free MOG peptides used at 17 and 19 fold excess respectively, were unable to suppress the clinical signs of EAE in their respective mouse strains (Figure 1, panels a and b). These results indicate that the agg Ig-chimeras display a broad effectiveness in reversing disease involving multiple epitopes and, most likely, diverse T cell specificities.

Example 2: Dendritic cells mediate the reversal of EAE by agg Ig-myelin chimeras.

In prior studies, it was demonstrated that both DCs produce IL-10 upon crosslinking of their FcγRs by agg Ig-PLP1 (21). Since neutralization of IL-10 during treatment of diseased mice with agg Ig-PLP1 restored clinical severity, it is possible that DCs play a critical role in the suppression of EAE (21). To evaluate the contribution of DCs to this form of peripheral T cell tolerance, an animal model where both antigen presentation and IL-10 secretion are compromised was needed. Since the delivery molecule belongs to the IgG2b subclass, an isotype that internalizes into APCs via FcγRs (20, 28) and induces IL-10 by the crosslinking of these receptors (21,22), mice deficient in FcγR expression would provide such a model. The FcγR

family includes three subfamilies that either employ an α chain that mediates both Ig binding and signaling (FcγR II) (29), or an α chain that mediates Ig binding and a γ chain responsible for signaling (FcγR I and III) (29). Mice in which the α chain of FcγR II was knocked out generated animals that were deficient for FcγR II (FcγR II $^{-/-}$) but displayed intact FcγR I and III (24). Also, gene targeted mutation of the common γ chain generated mice that were deficient in FcγR I and III (FcγR I, III $^{-/-}$), but not FcγR II expression (25). Genetic crossing of these two mutant strains generated mice deficient for all three types of FcγRs (FcγR I, II, III $^{-/-}$) (25).

Splenocytes from C57Bl/6 (a), FcγR II$^{-/-}$ (b), FcγR I, III$^{-/-}$ (c), and FcγR I, II, III$^{-/-}$ (d) were incubated with graded amounts of agg (white circles) or sol (black circles) Ig-MOG for 24 h. Subsequently, the supernatant was used to measure IL-10 production by ELISA. In order to determine the contribution of FcγR I and FcγR III in mediating IL-10 production, the following experiment was carried out (see insert in Figure 2, panel b): DCs from FcγR II$^{-/-}$ were incubated with 0.6 μM of agg Ig-chimera in the presence of 20 μg/ml 2.4G2 mAb (hatched bar) or 100 μg/ml mouse IgG (open bar), and IL-10 production was evaluated. Each point represents the mean of triplicate wells.

Using these mutant mice, FcγR I was identified as the major mediator of agg Ig-chimera induced IL-10 production by APCs (Figure 2). Indeed, splenocytes from C57Bl/6 or FcγR II$^{-/-}$ mice produced IL-10 upon incubation with agg Ig-MOG (Figure 2, panels a and b), while those from FcγR I, III$^{-/-}$ or FcγR I, II, III$^{-/-}$ mice did not, as IL-10 production remained at the background level seen with the negative control, soluble Ig-MOG (Figure 2, panels c and d). Similar results were obtained when DCs were used instead of splenocytes (data not shown).

These results indicate that FcγR I and/or FcγR III was responsible for binding agg Ig-MOG and triggering the IL-10 production by the APCs. However, blockade of FcγR III with 2.4G2 antibody, which is specific for both FcγR II and III (30) on cells lacking FcγR II, still allowed for equivalent IL-10 production indicating that FcγR I is most likely the major binder and thus, mediator of agg Ig-MOG induced IL-10 production (Figure 2b insert). Therefore, FcγR I, III $^{-/-}$ mice provide an appropriate model for adoptive transfer of wild type C57Bl/6 APCs to investigate the role of DCs in the suppression and reversal of EAE by agg Ig-chimeras.

Example 3: DCs mediate reversal of EAE in FcγR I, III $^{-/-}$ mice in conjunction with agg Ig chimera.

Accordingly, FcγR I, III $^{-/-}$ mice that had been induced for EAE with CNS homogenate were given C57Bl/6 DCs on day 5 post disease induction and were treated with agg Ig-MOG when clinical EAE became apparent.

Groups of FcγR I, III$^{-/-}$ mice (6-8 weeks of age) were induced for EAE with 6 mg of CNS homogenate. On day 5 following disease induction, the mice were adoptively transferred with 0.6 x 10^6 purified C57Bl/6 DCs (squares). Subsequently, the mice were treated intraperitoneally on days 9, 13 and 17 with 300 μg aggregated Ig-MOG per injection after disease induction. Groups of untreated mice that either received (black squares) or did not receive (black circles) DC transfer and mice that were treated with agg Ig-MOG but did not receive DC transfer (white circles) were included for comparison purposes. The clinical onset of disease was at day 5 in these experimental groups. Each point represents the mean clinical score of 6-8 mice.

As can be seen in Figure 3, the FcγR I, III $^{-/-}$ mice developed a severe disease (2.6 ± 0.2) in response to CNS homogenate (no transfer/no treatment group). In contrast to the wildtype

C57Bl/6 mice, these FcγR I and III$^{-/-}$ mice could not reverse their disease upon treatment with agg Ig-MOG (no transfer/ agg Ig-MOG group). However, if these mice were reconstituted with wild type DCs from C57Bl/6 mice prior to treatment with agg Ig-MOG, the severity of EAE was significantly reduced (mean maximal score of 1.1 ± 0.2) and the animals recovered by day 20 post disease induction (DCs Transfer/agg Ig-MOG group). Adoptive transfer of DCs without treatment with agg Ig-MOG had a marginal effect on disease manifestation (DC Transfer /No Treatment group).

These results indicate that DCs play a critical role in this form of peripheral tolerance and suppression of autoimmunity.

Example 4: DCs in conjunction with agg IG-MOG enhance IL-10 production and downregulate IL -12 production.

It has been previously shown that modulation of disease by the Ig-chimeras is due to tolerization of myelin-specific T cells and suppression of their proliferation as well as IFNγ production (26). In addition, IL-10 produced by APCs, upon binding of agg Ig-myelin chimeras, has been shown to play a critical role in T cell tolerization, and its neutralization by administration of anti-IL-10 antibodies restored disease severity (21). Suppression of EAE by agg Ig-myelin chimeras might operate through the direct binding of IL-10 to the T cells leading to inhibition of their proliferation (31-33). Alternatively, IL-10 may downregulate IL-12 production by the APCs and lead to defective T cell differentiation (31, 34, 35). To test this premise, DCs were assayed for production of IL-12 upon incubation with agg Ig-MOG and assessed whether any such IL-12 secretion would be subject to down-regulation by IL-10.

Twenty thousand purified DCs (a) were incubated with graded amounts of agg Ig-PLP1 for 24 hours, and the supernatant was then used to measure both IL-10 and IL-12 production by ELISA. In the Figure 4, panel b, the stimulation with agg Ig-PLP1 was carried out in the presence of 20 μg/ml anti-mouse IL-10 mAb antibody (2A5) or negative control rat IgG. Subsequently IL-10 (b) and IL-12 (c) were measured by ELISA. Each point or bar represents the mean ± SD of triplicate wells. The dotted line indicates the lower limit of cytokine detection in this ELISA.

As can be seen in Figure 4, DCs produced both IL-10 and IL-12 upon incubation with agg Ig-MOG. However, the secretion of IL-12 decreased as IL-10 production increased suggesting a regulation of IL-12 by IL-10. In fact, the addition of an anti-IL-10 mAb to the culture to neutralize IL-10 supported this view and allowed for IL-12 secretion at higher concentrations of agg Ig-chimera (Figure 4, panels b and c).

Example 5: The CD8α$^-$, but not CD8α$^+$, DC population is responsible for IL-10 production and reversal of EAE upon treatment with agg Ig-MOG.

While all DCs defined to date function as APCs, DCs are quite heterogeneous in their surface phenotypes (36 - 37). Recently, it has become clear that the different DC subtypes display different trafficking patterns (38) and contribute distinct developmental functions in T cell priming (7, 8). Since DCs produce IL-10 and mediate reversal of EAE upon treatment with agg Ig-MOG, the opportunity became available to investigate the function of the DC subsets in this model of peripheral T cell tolerance.

Initially, DCs were separated into CD8α$^+$ and CD8α$^-$ populations and tested for both IL-10 and IL-12 production upon incubation with agg Ig-MOG. 50,000 purified CD8α$^+$ (panels a

and c, Figure 5) or CD8α⁻ (panels b and d, Figure 5) DCs were incubated with graded amounts of agg (white circles) or sol (black circles) Ig-PLP1 for 24 h. Subsequently, the supernatant was used to measure both IL-10 (panels a and b) and IL-12 (panels c and d) production by ELISA. Each point represents the mean of triplicate wells. The results shown in panels A - D in Figure 5 are representative of 4 experiments. The dotted line indicated the lower limit of cytokine detection in this ELISA

As illustrated in Figure 5, the CD8α^- DCs produced IL-10 while CD8α^+ cells secreted IL-12 upon crosslinking of their FcγRs by agg Ig-PLP1. The production of IL-12 by CD8α^+ DCs was also inducible by sol Ig-chimeras, possibly indicating that binding of monomeric IgG to FcγRs is sufficient to trigger IL-12 secretion. Alternatively, some sol Ig-MOG might have aggregated during culture at 37°C. If this is the case, IL-12 induction requires only a small amount of agg Ig relative to that required for the induction of IL-10 (Figure 4).

Example 6: CD8α^- DCs are unable to promote production of IFNγ by T cells.

Since the CD8α^- DCs produced IL-10 upon incubation with agg Ig-chimeras, T cells engaged by these APCs through peptide presentation should be tolerized rather than stimulated (31, 32). To test this premise, the PLP1-specific Th0 clone, TCC-PLP1-1B10 (21) (see also "Materials and Methods"), was incubated with CD8α^- DCs in the presence of sol or agg Ig-PLP1 and both IL-10 production by the APCs and IFNγ secretion by the T cells were measured. For comparison purposes, bulk and CD8α^+ DCs were included in these experiments.

CD8α^- dendritic cells are unable to promote the production of IFNα by T cells. Purified CD8α^+ (Fig. 6, panels a and b), CD8α^- (Fig. 6, panels c and d) and unseparated CD11c$^+$ (Fig. 6, panels e and f) DCs (5×10^4 cells/well) were incubated with graded amounts of agg (black

circles) or sol (white circles) Ig-PLP1 for 1 h. Subsequently, TCC-PLP1-1B10 Th0 cells (5 x10^4 cells/well) were added, and the incubation was continued for 24 h. IL-10 (Fig. 6, panels a, c and e) and IFNα (Fig. 6, panels b, d and f) production in the same culture well were then measured by ELISA from 100 μl of culture supernatant. The insert in panel d, measuring IL-4 secretion, indicates that the absence of IFNγ production by the T cells upon stimulation with CD8α^- DCs and Ig-PLP1 was not due to inadequate antigen presentation. Each point represents the mean of triplicate wells. The dotted line indicates the lower limit of cytokine detection in this ELISA.

As can be seen in Figure 6, panels a and b, CD8α^+ DCs, which do not produce IL-10 upon incubation with agg or sol Ig-PLP1, activated the Th0 clone to produce IFNγ through presentation of PLP1 peptide. In contrast, CD8α^- DCs, which produce IL-10 upon incubation with agg Ig-PLP1 (Figure 6, panel c), were not able to drive IFNγ production by the Th0 clone (Figure 6, panel d). Surprisingly, incubation with sol Ig-PLP1, which does not crosslink FcγRs or trigger IL-10 production by the CD8α^- DCs (Figure 6, panel c), was unable to stimulate the Th0 clone for IFNγ production (Figure 6, panel d). The lack of IFNγ was not due to defective presentation of sol Ig-PLP1 as the Th0 clone was able to produce IL-4 in response to such stimulation (Figure 6, panel d insert). Nevertheless, the IL-10 produced by the CD8α^- subset within the bulk DC population (Figure 6, panel e) suppressed the capacity of the CD8α^+ subset to drive IFNγ production by the T cells (Figure 6, panel f), a phenomenon that further argues for the toleragenic function of CD8α^- DCs.

Overall, the results of this *in vitro* experiment with a Th0 clone demonstrate that CD8α^- DCs do not support the polarization of T cells into IFNγ producing cells and can actively antagonize the activation of Th1 cells through IL-10 production.

Example 7: CD8α⁻ DCs tolerize pathogenic myelin specific Th1 cells and enhance peripheral tolerance and bystander suppression.

The ability of CD8α^- DCs to mediate T cell tolerance *in vivo* was analyzed. To this end, CD8α^- DCs were adoptively transferred into FcγR I, III$^{-/-}$ mice and tested for reversal of EAE upon treatment with agg Ig-MOG.

CD8α^- wild type dendritic cells restore agg Ig-MOG mediated reversal of EAE in FcγR I, III$^{-/-}$ mice. Groups of FcγR I, III$^{-/-}$ mice (6-8 weeks of age) were induced for EAE with 6 mg of CNS homogenate. On day 5 following disease induction, the mice were given intraperitoneally 3 x 10^5 (a) or 6 x10^5 (b) purified C57Bl/6 CD8α^- (black circles) or CD8α^+ DCs (white circles). Subsequently, the mice were treated intraperitoneally with 300 μg agg Ig-MOG on days 9, 13 and 17 after disease induction. A group of mice that were treated with agg Ig-MOG but did not receive cell transfer (white triangles) was included for comparison purposes. The clinical onset of disease was at day 5 in these experimental groups. Each point represents the mean clinical score of 6-8 mice.

The results illustrated in Figure 7, panels a and b, indicate that transfer of either 3 or 6 x 10^5 C57Bl/6 CD8α^- DCs into FcγR I and III$^{-/-}$ mice with ongoing EAE allowed for the reversal of paralysis when the mice were treated with agg Ig-MOG. However, mice adoptively transferred with CD8α^+ DCs, instead of the CD8α^- population, were unable to suppress the disease and had clinical scores similar to those of mice that did not receive any cell transfer.

These results indicate that CD8α^- DCs are responsible for tolerization of pathogenic myelin-specific Th1 T cells and thus, play a critical role in this form of peripheral tolerance coupled with IL-10 bystander suppression.

Example 8: CD8α^+, but not CD8α^-, DCs promote development of EAE.

It has been previously shown that DCs loaded with antigenic peptide induce immune responses without the requirement for adjuvant (3). This regimen provided a useful approach to test the intrinsic toleragenic function of the CD8α^- DCs. Accordingly, CD8α^- and CD8α^+ DCs were loaded with an excess of PLP1 peptide *in vitro* and injected into mice according to a regimen defined to induce EAE (39).

Purified CD8α^+ (black circles) or CD8α^- (white triangles) DCs were pulsed over night with 50 μg/ml PLP1 peptide, washed, resuspended in PBS and injected (3 x 10^5/mouse) into the hind limb footpads of SJL/J mice. 6 and 24 h later, the mice were given intravenously 200 ng of B. pertussis toxin. The clinical onset of disease was at day 6 in these experimental groups, and each point represents the mean clinical score of 5 mice.

Daily analysis of paralytic scores indicated that CD8α^+, but not CD8α^- DCs, induced EAE (Figure 8). These results demonstrate that PLP1-reactive T cells were available but were unresponsive to PLP1 peptide when it was presented by these CD8α^- DCs as activation and differentiation into Th1 effectors that cause disease did not occur (7, 8).

Example 9: CD8α^-CD4$^+$, but not CD8α^-CD4$^-$, DCs produce IL-10 and mediate T cell tolerance and reversal of EAE upon treatment with Ig-MOG.

The CD8α^- subset of splenic DCs is comprised of two phenotypically different populations based upon the expression of CD4 (37). However, whether these DC subsets, CD8α^-CD4$^+$ and CD8α^-CD4$^-$, display distinct biologic functions has not yet been clarified. Herein, the two subsets were separated from splenic DCs and tested for IL-10 production as well as reversal of EAE upon treatment with agg Ig-MOG.

IL-10 production by purified (a) CD11c^{+}CD8α^{-}CD4^{+}, (b) CD11c^{+}CD8α^{-}CD4^{-}, and (c) CD11c^{+}CD8α^{+} DCs were measured by intracellular cytokine staining. Accordingly, DCs (1 x 10^{6}/well) were incubated with 0.6 μM agg Ig-PLP1 for 24 h and brefeldin A (10 μg) was added to the wells during the last 8 h of incubation in order to block cytokine secretion. Subsequently, the cells were harvested and stained with anti-CD11c-APC and FITC-labeled anti-CD8α or anti-CD4 mAb. The cells were then fixed, permeabilized and intracellular stained with PE-conjugated anti-IL-10 (thick lines) or isotype matched (doted lines) mAb. Cells incubated with media without the addition of Ig-PLP1 (thin lines) and intracellular stained with anti-IL-10-PE mAb were included for control purposes. Histogram plots are shown representing the intensity of IL-10 expression by the indicated DCs population. The results shown are representative of 4 experiments.

In Figure 9 (panels d and e), groups of FcγR I, III$^{-/-}$ mice (6-8 weeks of age) were induced for EAE with 6 mg of CNS homogenate and on day 5 the mice were given intraperitoneally. 3x 10^{5} (d) or 6 x10^{5} (e) purified C57Bl/6 CD8α^{-}CD4^{+} (closed circles) or CD8α^{-}CD4^{-} (open circles) DCs. Subsequently, the mice were treated intraperitoneally with 300 μg agg Ig-MOG on days 9, 13 and 17 post disease induction. A group of untreated mice that received 0.6 x 10^{6} CD8α^{-}CD4^{+} DCs (open triangles) was included for comparison purposes. The clinical onset of disease was at day 5 in these experimental groups. Each point represents the mean clinical score of 6-8 mice.

The results illustrated in Figure 9 indicate that CD8α^{-}CD4^{+}, but not CD8α^{-}CD4^{-} , DCs produce IL-10 upon incubation with agg Ig-MOG as tested by intracellular cytokine staining (Figure 9, panels a and b). IL-10 production was not observed with the CD8α^{+} subset that was used as a negative control (Figure 9, panel c).

Subsequently, the two subsets were adoptively transferred into FcγR I, III $^{-/-}$ mice with ongoing clinical EAE and tested for reversal of paralysis upon treatment with agg Ig-MOG. The results indicate that $CD8\alpha^{-}CD4^{+}$, but not $CD8\alpha^{-}CD4^{-}$, DCs were able to mediate tolerance and reverse the disease upon treatment with agg Ig-MOG (Figure 9, panels d and e). Indeed, the mice transferred with 3 or 6 x 10^5 $CD8\alpha^{-}CD4^{+}$ DCs had a mean maximal disease severity of 0.9 ± 0.3 and fully recovered by day 18 post disease induction. Those transferred with equivalent numbers of $CD8\alpha^{-}CD4^{-}$ DCs had a mean maximal disease severity of 2.6 ± 0.3 and did not recover from their paralysis during the 21 day period of clinical assessment. The reversal of EAE requires treatment with agg Ig-MOG because adoptive transfer of 6 x 10^5 $CD8\alpha^{-}CD4^{+}$ DCs did not confer reduction of the severity of disease or recovery from paralysis when the animal were not given agg Ig-MOG. These results identify the $CD8\alpha^{-}CD4^{+}$ DC subset as the toleragenic population among mature splenic DCs.

The following references 1- 50 are hereby incorporated by reference in their entireties:

1. Steinman, R.M. 1991. The dendritic cell system and its role in immunogenicity. Annu. Rev. Immunol. 9:271-296.

2. Macatonia, S.E., P.M. Taylor, S.C. Knight, and B.A. Askonas. 1989. Primary stimulation by dendritic cells induces antiviral proliferative and cytotoxic T cell response in vitro. *J. Exp. Med.* 169:1255-1264.

3. Inaba, K., J.P. Metlay, M.T. Crowley, and R.M. Steinman. 1990. Dendritic cells pulsed with protein antigens in vitro can prime antigen-specific, MHC-restricted T cells in situ. *J. Exp. Med.* 172: 631-640.

4. Sornasse, T., V. Flamand, G. De Becker, H. Bazin, F. Tielemans, K. Thielemans, J. Urbain, O. Leo, and M. Moser. 1992. Antigen-pulsed dendritic cells can efficiently induce an antibody response in vivo. *J. Exp. Med.* 175:15-21.

5. Kronin, V., K. Winkel, G. Shss, A. Kelso, W. Heath, J. Kirberg, H. von Boehmer, and K. Shortman. 1996. A subclass of dendritic cells regulates the response of naïve CD8 T cells by limiting their IL-12 production. *J. Immunol.* 157:3819-3827.

6. Rissoan, M.C., V. Soumelis, N. Kadowaki, G. Grouard, F. Briere, R. de Waal Malefyt, and Y.J. Liu. 1999. Reciprocal control of T helper cell and dendritic cell differentiation. *Science* 283:1183-1186.

7. Maldonado-Lopez, R., T. De Smedt, P. Michel, J. Godfroid, B. Pajak, C. Heirman, K. Thielemans, O.Leo, J. Urbain, and M. Moser. 1999. CD8α^+ and CD8α^- subclasses of dendritic cells direct the development of distinct T helper cells in vivo. *J. Exp. Med.* 189:587-592.

8. Pulendran, B., J.L. Smith, G. Caspary, K. Brasel, D. Pettit, E. Maraskovsky, and C.R. Maliszewski. 1999. Distinct Dendritic cell subsets differentially regulate the class of immune response in vivo. *Proc. Natl. Acad. Sci. USA* 96:1036-1041.

9. Albert, M.L., B. Sauter, and N. Bhardwaj. 1998. Dendritic cells acquire antigen from apoptotic cells and induce class I-restricted CTLs. *Nature* 392:86-89.

10. Sauter, B., M.L. Albert, L. Francisco, M. Larsson, S. Somersan, and N. Bhardwaj. 2000. Consequences of cell death: exposure to necrotic tumor cells, but not primary tissue cells or apoptotic cells, induces the maturation of immunostimulatory dendritic cells. *J. Exp. Med.* 191:423-433.

11. Bevan, M.J. 1976. Cross-priming for a secondary cytotoxic response to minor H antigens with H-2 congenic cells which do not cross-react in the cytotoxic assay. *J. Exp. Med.* 143: 1283-1288.

12. den Haan, J.M., S.M. Lehar, and M.J. Bevan. 2000. $CD8^+$ but not $CD8^-$ dendritic cells cross-prime cytotoxic T cells in vivo. *J. Exp. Med.* 192:1685-1695.

13. Sigal, J.L., S. Crotty, R. Anidino, and K.L. Rock. 1999. Cytotoxic T-cell immunity to virus-infected non-haematopoietic cells requires presentation of exogenous antigen. *Nature* 398:77-80.

14. Berard, F., P. Blanco, J. Davoust, E.M. Neidhart-Berard, M. Nouri-Shirazi, N. Taquet, D. Rimoldi, J.C. Cerottini, J. Banchereau, and A.K. Paluka. 2000. Cross-priming of naïve CD8 T cells against melanoma antigens using dendritic cells loaded with killed allogeneic melanoma cells. *J. Exp. Med.* 192:1535-1543.

15. Subklewe, M., C. Paludan, M.L. Tsang, K. Mahnke, R.M. Steinman, and C. Mhnz. 2001. Dendritic cells cross present latency gene products from Epstein-Barr virus-transformed B cells and expand tumor-reactive $CD8^+$ killer T cells. *J. Exp. Med.* 193:405-411.

16. Inaba, M., K. Inaba, M. Hosono, T. Kumamoto, T. Ishida, S. Muramatsu, T. Masuda, and S. Ikehara. 1991. Distinct mechanism of neonatal tolerance induced by dendritic cells and thymic B cells. *J. Exp. Med.* 173:549-559.

17. Khoury, S.J., L. Gallon, W. Chen, K. Betres, M.E. Russell, W.W. Hancock, C.B. Carpenter, M.H. Sayegh, and H.L. Weiner. 1995. Mechanisms of acquired thymic tolerance in

experimental autoimmune encephalomyelitis: thymic dendritic-enriched cells induce specific peripheral T cell unresponsiveness in vivo. *J. Exp. Med.* 182: 357-366.

18. Broker, T., M. Riedinger, and K. Karjalainen. 1997. Targeted expression of major histocompatibility complex (MHC) class II molecules demonstrates that dendritic cells can induce negative but not positive selection of thymocytes in vivo. *J. Exp. Med.* 185:541-50.

19. Steinman, R.M., S. Turley, I. Mellman, and K. Inaba. 2000. The induction of tolerance by dendritic cells that have captured apoptotic cells. *J. Exp. Med.* 191:411-416.

20. Legge, K. L., B. Min, N. T. Potter, and H. Zaghouani. 1997. Presentation of a T cell receptor antagonist peptide by immunoglobulins ablates activation of T cells by a synthetic peptide or proteins requiring endocytic processing. *J. Exp. Med.* 185:1043-1053.

21. Legge, K.L., B. Min, J. J. Bell, J.C. Caprio, L. Li, R.K. Gregg, and Habib Zaghouani. 2000. Coupling of peripheral tolerance to endogenous interleukin 10 promotes effective modulation of myelin-activated T cells and ameliorates experimental allergic encephalomyelitis. *J. Exp. Med.* 191:2039-2051.

22. Legge, K. L., J. J. Bell, L. Li, R. Gregg, J. C. Caprio, and H. Zaghouani. 2001. Multi-modal antigen specific therapy for autoimmunity. *Intern. Rev. Immunol* 20:593-611.

23. Bernard, C.C.A., T.G. Johns, A. Slavin, M. Ichikawa, C. Ewing, J. Lui, and J. Bettadapura. 1997. Myelin oligodendrocyte glycoprotein: a novel candidate autoantigen in multiple sclerosis. J. Mol. Med. 75:77-88.

24. Takai, T., M. Ono, M. Hikida, H. Ohmori, and J.V. Ravetch. 1996. Augmented humoral and anaphylactic responses in Fc gamma RII-deficient mice. *Nature* 379:346-349.

25. Takai, T., M. Li, D. Sylvestre, R. Clynes, and R.V. Ravetch. 1994. FcR gamma chain deletion results in pleiotrophic effector cell defects. *Cell* 76: 519-529.

26. Tuohy, V. K., Z. Lu, R. A. Sobel, R. A. Laursen, and M.B. Lees. 1989. Identification of an encephalitogenic determinant of myelin proteolipid protein for SJL mice. J. Immunol. 142:1523-1527.

27. Romani, N., N. Bhardwaj, M. Pope, F. Koch, W. J. Swiggard, U.O. Doherty, M.D. Witmer-Pack, L. Hoffman, G. Schuler, K. Inaba, and R.M. Steinman. 1996. Dendritic cells. In Weirs Handbook of Experimental Immunology. L.A. Herzenberg, D. Weir, L.A. Herzenberg, and C. Blackwell, editors. Blackwell Science, Cambridge, MA 156.1-156.14.

28. Zaghouani, H., R.M. Steinman, R. Nonacs, H. Shah, W. Gerhard, and C. Bona. 1993. Presentation of a viral T cell epitope expressed in the CDR3 region of a self immunoglobulin molecule. Science. 259:224-227.

29. Daeron, M. 1997. Fc receptor biology. Annu. Rev. Immunol. 15: 203-234.

30. Unkeless, J.C. 1979. Characterization of a monoclonal antibody directed against mouse macrophage and lymphocyte Fc receptors. J. Exp. Med. 150:580-596.

31. Moore, K.W., R. de Waal Malefyt, R. L. Coffman, and A. O'Garra. 2001. Interleukin-10 and the interleukin-10 receptor. Annu. Rev. Immunol. 19:683-765.

32. Macatonia, S.E., T.M. Doherty, S.C. Knight, and A. O'Garra. 1993. Differential effect of interleukin 10 on dendritic cell-induced T cell proliferation and IFN□ production. J. Immunol. 150:3755-3765.

33. Joss, A., M. Akdis, A. Faith, K. Blaser, and C.A. Akdis. 2000. IL-10 directly acts on T cells by specifically altering the CD28 co-stimulation pathway. Eur J. Immunol. 30:1683-1690.

34. Groux, H., M. Bigler, J.E. de Vries, and M.G. Roncarolo. 1996. Interleukin-10 induces a long term antigen-specific anergic state in human $CD4^+$ T cells. J. Exp. Med. 184:19-29.

35. Steinbrink, K., M. Wolfl, H. Jonuleit, J. Knop, and A.H. Enk. 1997. Induction of tolerance by IL-10 treated dendritic cells. J. Immunol. 159:4772-4780.

36. Vermec, D., M. Zorbas, R. Scollay, D.J. Saunders, C.F. Ardavin, L. Wu, and K. Shortman. 1992. The surface phenotype of dendritic cells purified from mouse thymus and spleen;

investigation of the CD8 expression by a subpopulation of dendritic cells. J. Exp. Med. 176:47-58

37. Vermec, D., J. Pooley, H. Hochrein, L. Wu, and K. Shortman. 2000. CD4 and CD8 expression by dendritic cell subtypes in mouse thymus and spleen. J. Immunol. 164:2978-2986.

38. Pulendran, B., J. Lingappa, M.K. Kennedy, J. Smith, M. Teepe, A. Rudensky, C.R. Maliszewski, and E. Maraskovsky. 1997. Develpomental pathways of dendritic cells in vivo: distinct function, phenotype, and localization of dendritic cell subsets in FLT3 ligand-treated mice. J. Immunol. 159:2222-2231.

39. Dittel, B.N., I. Visintin, R.M. Merchant, and C.A. Janeway, Jr. 1999. Presentation of the self antigen myelin basic protein by dendritic cells leads to experimental autoimmune encephalomyelitis. J. Immunol. 163:32-39.

40. Brumeanu, T. D., W. J. Swiggard, R. M. Steinman, C. A. Bona, and H. Zaghouani. 1993. Efficient loading of identical viral peptide onto class II molecules by antigenized immunoglobin and influenza virus. J. Exp. Med. 178:1795-1799.

41. Maldonado-Lopez, R., C. Maliszewski, J. Urbain, and M. Moser. 2001. Cytokines regulate the capacity of CD8alpha(+) and CD8alpha(-) dendritic cells to prime Th1/Th2 cells in vivo. J. Immunol.167:4345-4350.

42. Dhodapkar, M.V., R.M. Steinman, J. Krasovsky, C. Mhnz, and N. Bhardwaj. 2001. Antigen-specific inhibition of effector T cell function in humans after injection of immature dendritic cells. J. Exp. Med. 193:233-238.

43. Jonuleit, H., E. Schmitt, G. Schuler, J. Knop, and A.H. Enk. 2000. Induction of interleukin-10-producing, nonproliferating $CD4^+$ T Cells with regulatory properties by repetitive stimulation with allogeneic immature human dendritic cells. J. Exp. Med. 192:1213-1222.

44. Huang, F.P., N. Platt, M. Wykes, J.R. Major, T.J. Powell, C.D. Jenkins, and G.G. MacPherson. 2000. A discrete subpopulation of dendritic cells transports apoptotic intestinal epithelial cells to T cell areas of mesenteric lymph nodes. J. Exp. Med. 191:435-443.

45. Menges, M., S. Rößner, C. Voigtländer, H. Schindler, N. A. Kukutsch, C. Bogdan, K. Erb, G. Schuler, and M. B. Lutz. 2002. Repetitive injections of dendritic cells matured with tumor necrosis factor α induce antigen-specific protection of mice from autoimmunity. J. Exp. Med. 195:15-21.

46. Hawiger, D., K. Inaba, Y. Dorsett, M. Guo, K. Mahnke, M. Rivera, J. V. Ravetch, R. M. Steinman, and M. C. Nussensweig. 2001. Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions in vivo. J. Exp. Med. 194:769-779.

47. Cua, D. J., B. Hutchins, D. M. LaFace, S. A. Stohlman, and R. L. Coffman. 2001. Central nervous system expression of IL-10 inhibits autoimmune encephalomyelitis. J. Immunol. 166:602-608.

48. Suss, G., and K. Shortman. 1996. A subclass of dendritic cells kills CD4 T cells via Fas/Fas-ligand-induced apoptosis. J. Exp. Med. 183:1789-1796.

49. Jerne, NK. 1974. Towards a network theory of the immune system. Ann. Immunol. 125C:373-389.

50. Anderson, A.C., L.B. Nicholson, K.L. Legge, V. Turchin, H. Zaghouani, and V.K. Kuchroo. 2000. High frequency of autoreactive myelin proteolipid protein-specific T cells in the periphery of naïve mice: mechanisms of selection of the self-reactive repertoire. J. Exp. Med. 191:761-770.

Claims:

1. A method of tolerizing autoreactive T cells in a subject suffering from an autoimmune disorder comprising administering dendritic cells to the subject in conjunction with a pharmaceutically effective amount of an aggregated immunoglobulin or portion thereof wherein the immunoglobulin or portion thereof is linked to a peptide or portion thereof wherein the peptide or portion thereof is associated with the autoimmune disorder.

2. The method of claim 1 wherein the dendritic cells are administered together with or in close proximity with aggregated Ig-peptide.

3. The method of claim 1 wherein the autoimmune disorder is multiple sclerosis and the T cells are myelin T lymphocytes.

4. The method of claim 1 wherein the tolerization of autoreactive T cell includes tolerization and suppression of T cells.

5. The method of claim 1 wherein the DCs are CD8α- dendritic cells.

6. The method of claim 5 wherein the subject is administered, in conjunction with the dendritic cells, a composition from the group consisting of agg Ig-MOG and agg Ig-PLP1.

7. The method of claim 6 wherein the method results in enhanced IL-10 production and decreased IL-12 production.

8. The method of claim 7 wherein the method results in increased bystander regulation and increased peripheral tolerance.

9. The method of claim 7 wherein the CD8α- dendritic cells are CD8α-CD4$^+$ cells.

10. A method of treating an immune disorder comprising administering to a patient a therapeutically effective amount of dendritic cells in conjunction with an immunoglobulin-peptide construct.

11. The method of claim 10 wherein the immune disorder is multiple sclerosis and further comprises administering a composition selected from the group consisting of agg Ig-MOG and agg IG-PLP1.

12. The method of claim 11 wherein the dendritic cells are CD8α-CD4$^+$ dendritic cells.

13. The method of claim 12 wherein the production of IL-10 is significantly enhanced.

14. The method of claim 12 wherein the production of IL-12 is significantly decreased.

15. The method of claim 12 wherein the administration of dendritic cells tolerize autoreactive T cells.

16. The method of claim 12 wherein the dendritic cells tolerize pathogenic Th1 cells and enhance IL-10 production.

17. Use of a composition for the treatment of an autoimmune disorder wherein the composition comprises a pharmaceutically effective amount dendritic cells wherein the dendritic cells are administered to a patient suffering from the autoimmune disorder in conjunction with a pharmaceutically effective amount of an immunoglobulin or portion thereof, linked to one or more peptides derived from self antigens.

18. The use of claim 17 wherein the autoimmune disorder is multiple sclerosis.

19. The use of claim 18 wherein the immunoglobulin or portion thereof is selected from the group consisting of agg Ig-MOG and agg Ig-PLP1 and the dendritic cells are $CD8\alpha^{-}CD4^{+}$ dendritic cells.

20. The use of claim 17 wherein the autoimmune disorder is a T cell mediated autoimmune disorder selected from the group consisting of multiple sclerosis, type 1 diabetes and rheumatoid arthritis.

Fig. 1

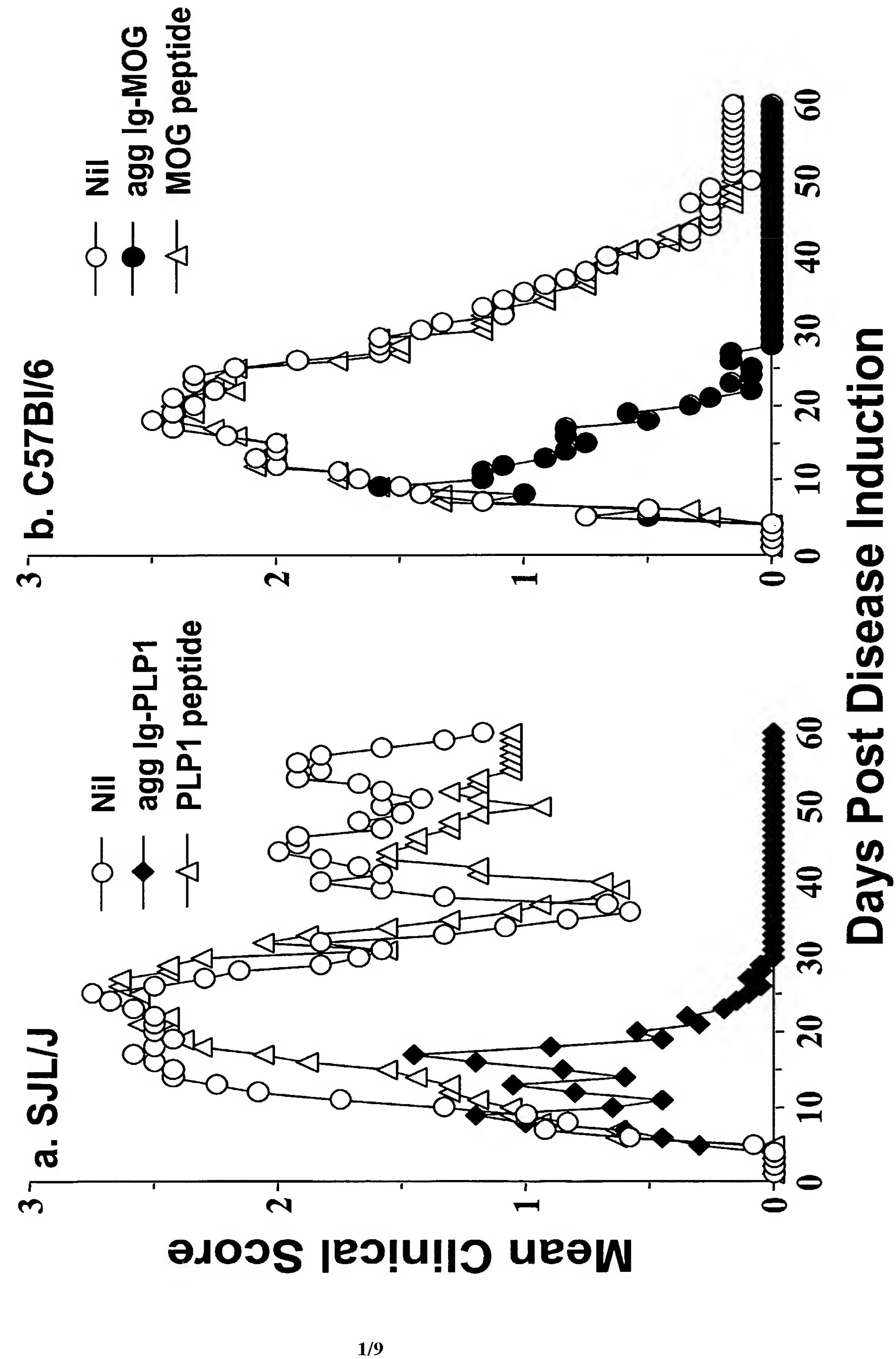

a. SJL/J
Nil
agg Ig-PLP1
PLP1 peptide
b. C57Bl/6
Nil
agg Ig-MOG
MOG peptide
Mean Clinical Score
Days Post Disease Induction
0
1
2
3
0
10
20
30
40
50
60

Fig. 2

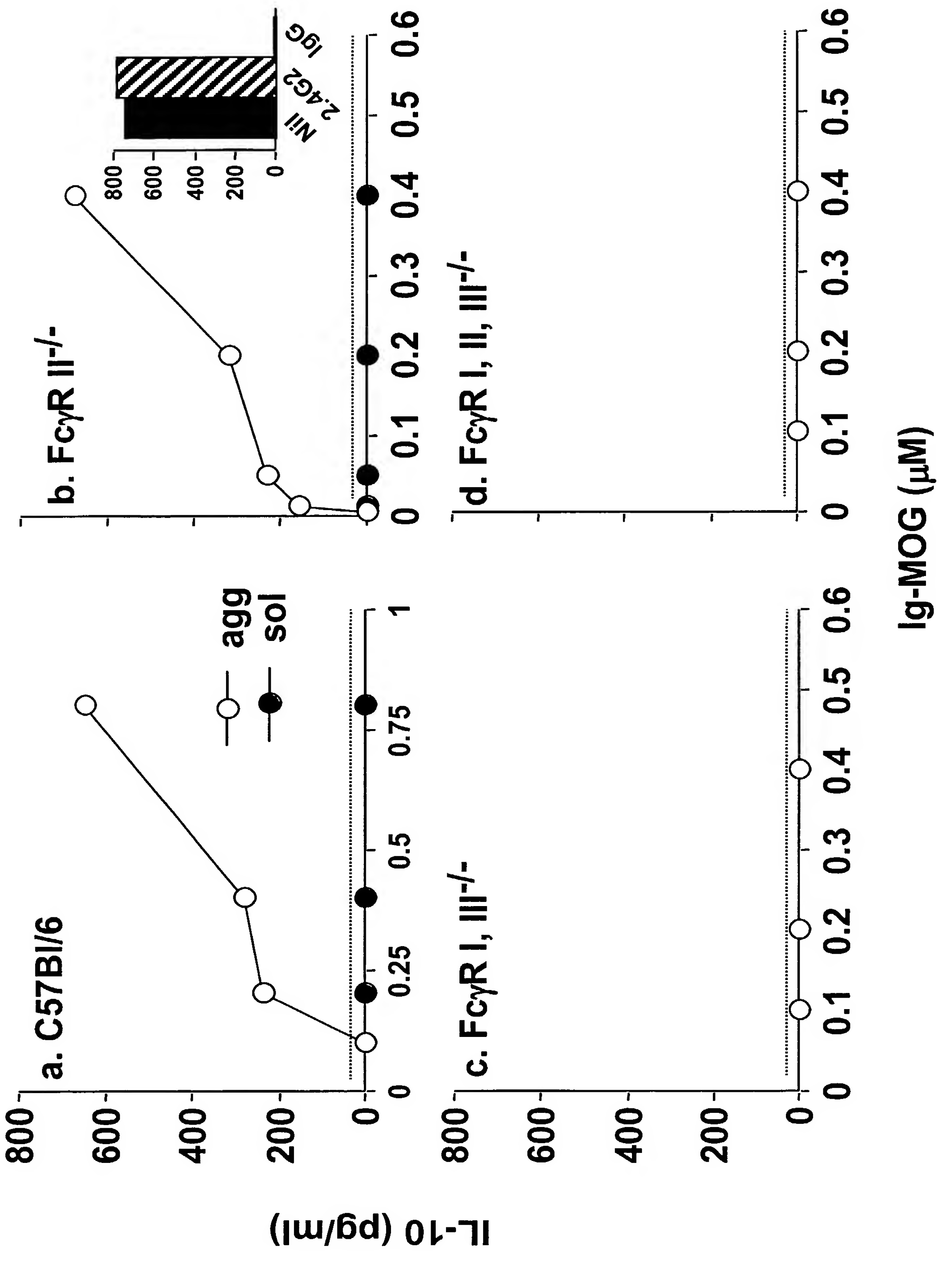

a. C57Bl/6
b. FcγR II-/-
c. FcγR I, III-/-
d. FcγR I, II, III-/-
agg
sol
IL-10 (pg/ml)
Ig-MOG (μM)
800
600
400
200
0
0.25
0.5
0.75
1
0.1
0.2
0.3
0.4
0.5
0.6
Nil
2.4G2
IgG

Fig. 3

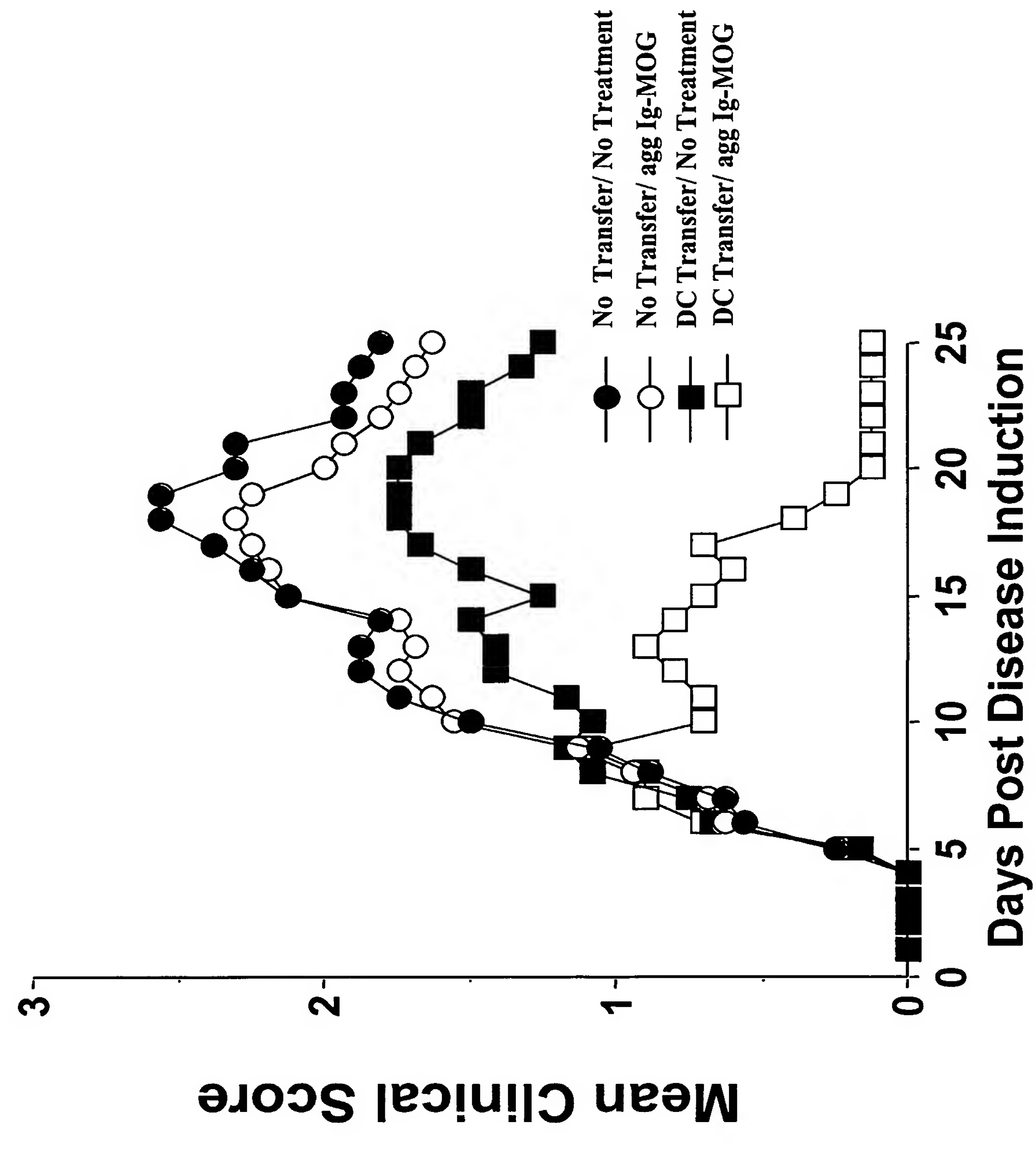
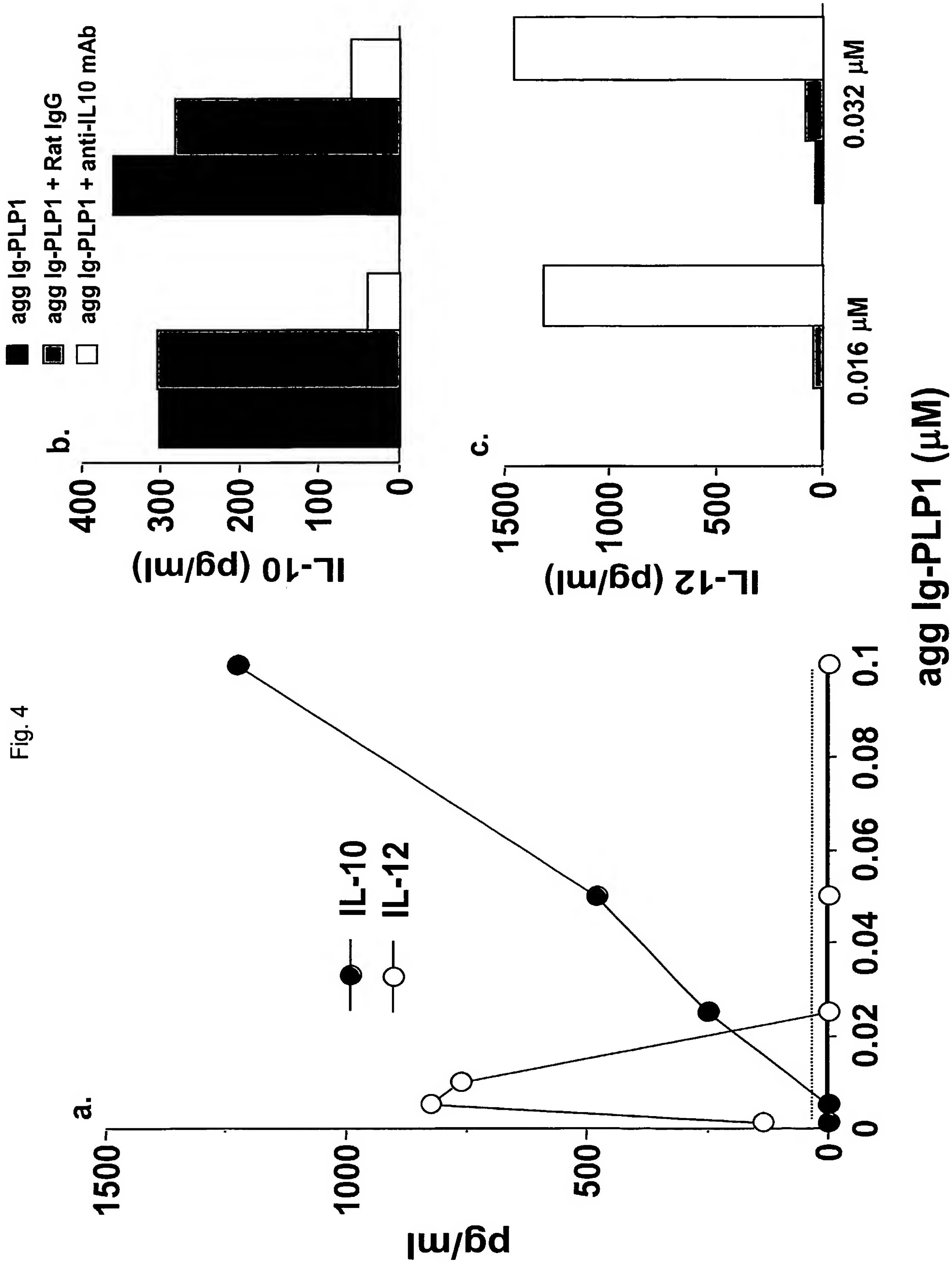

No Transfer/ No Treatment
No Transfer/ agg Ig-MOG
DC Transfer/ No Treatment
DC Transfer/ agg Ig-MOG
Mean Clinical Score
0
1
2
3
Days Post Disease Induction
0
5
10
15
20
25

Fig. 4


a.
pg/ml
1500
1000
500
0
0
0.02
0.04
0.06
0.08
0.1
IL-10
IL-12
b.
IL-10 (pg/ml)
400
300
200
100
0
agg Ig-PLP1
agg Ig-PLP1 + Rat IgG
agg Ig-PLP1 + anti-IL10 mAb
c.
IL-12 (pg/ml)
1500
1000
500
0
0.016 μM
0.032 μM
agg Ig-PLP1 (μM)

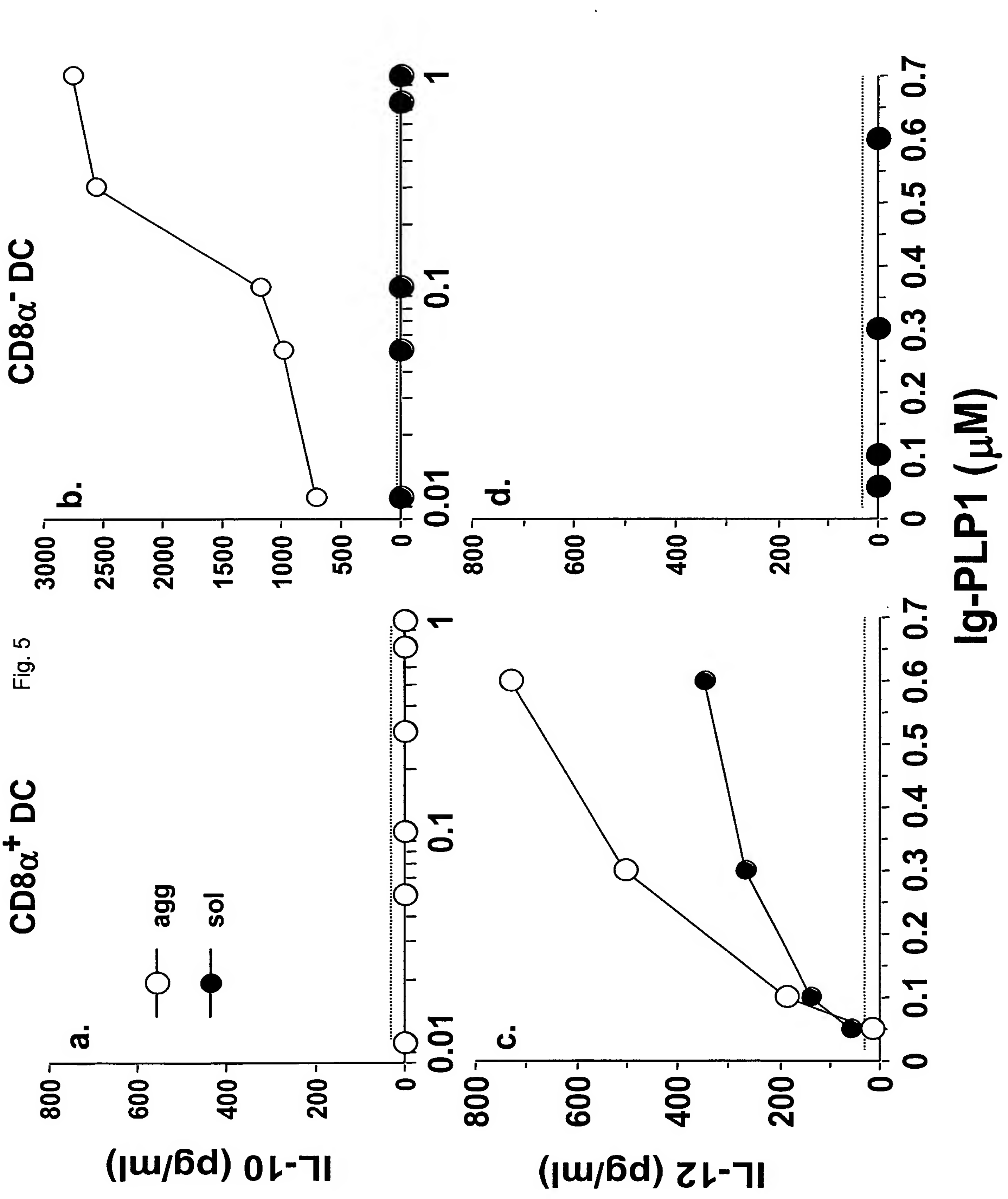

CD8α⁺ DC
CD8α⁻ DC
a.
b.
c.
d.
agg
sol
IL-10 (pg/ml)
IL-12 (pg/ml)
Ig-PLP1 (μM)
0.01
0.1
1
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0
200
400
600
800
0
500
1000
1500
2000
2500
3000

Fig. 5

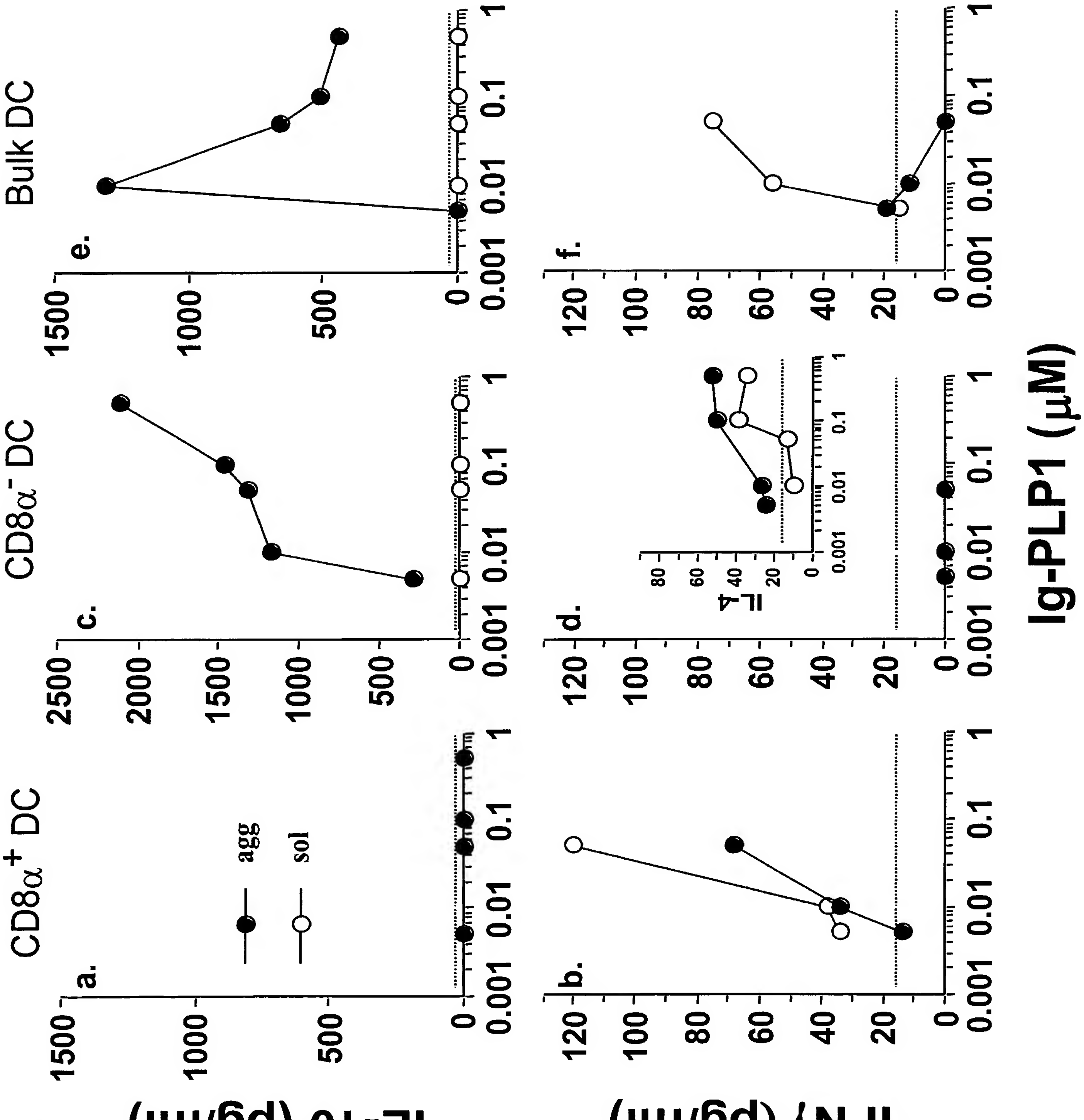
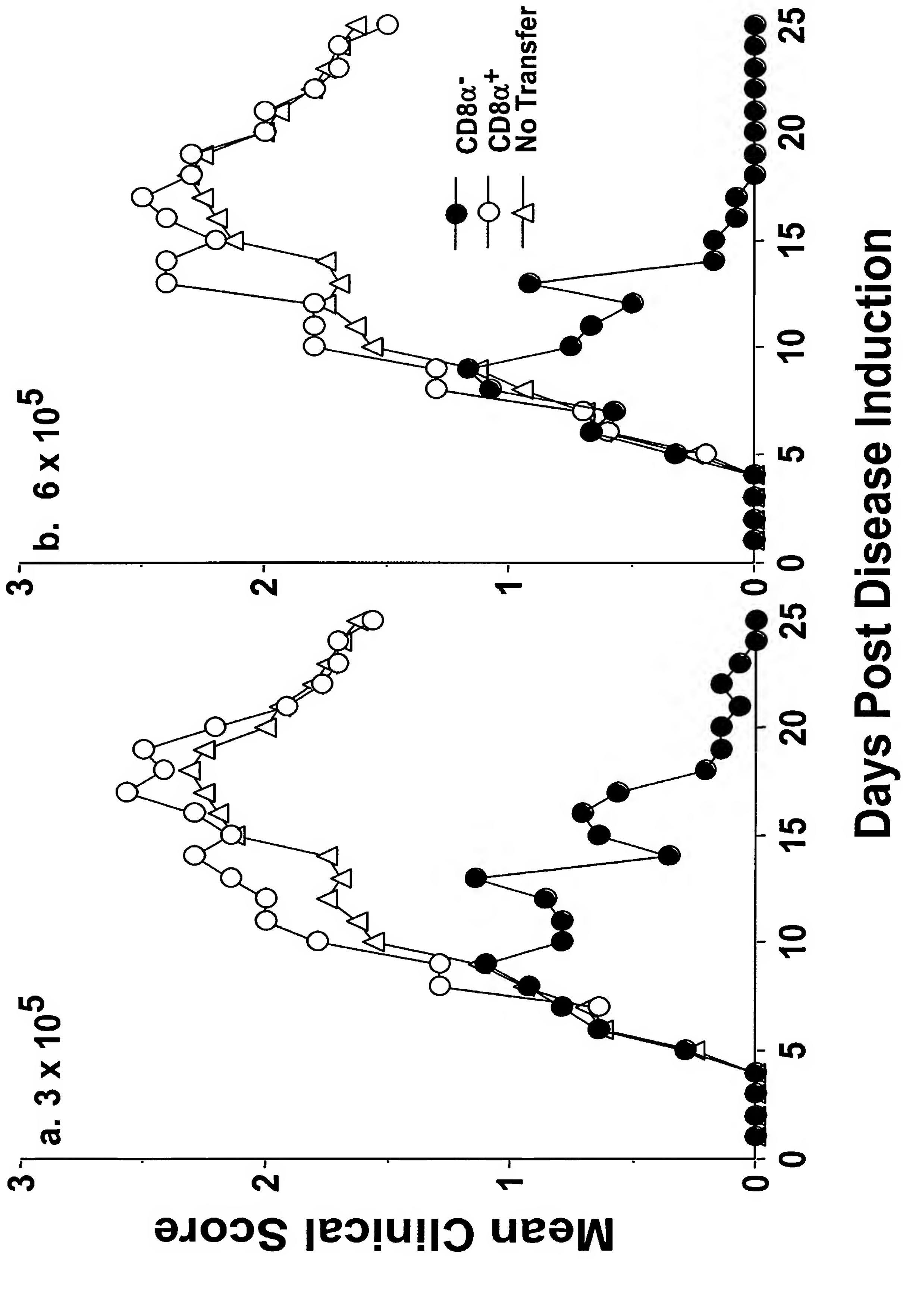

CD8α+ DC
CD8α- DC
Bulk DC
a.
b.
c.
d.
e.
f.
agg
sol
IL-10 (pg/ml)
IFNγ (pg/ml)
IL-4
Ig-PLP1 (μM)

Fig. 6

Fig. 7



a. 3 x 10^5
b. 6 x 10^5
Mean Clinical Score
Days Post Disease Induction
CD8α⁻
CD8α⁺
No Transfer
0
1
2
3
5
10
15
20
25

Fig. 8

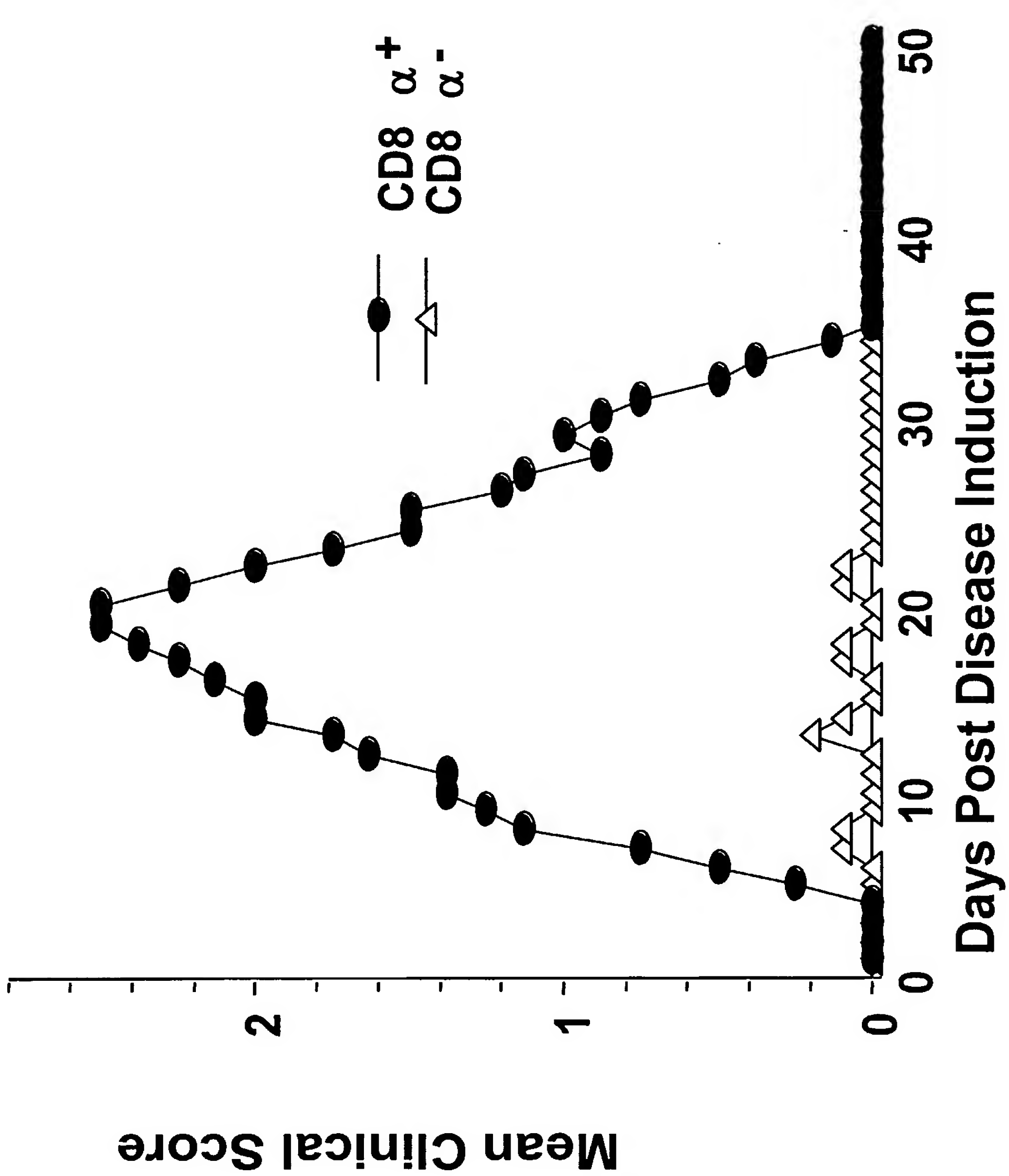
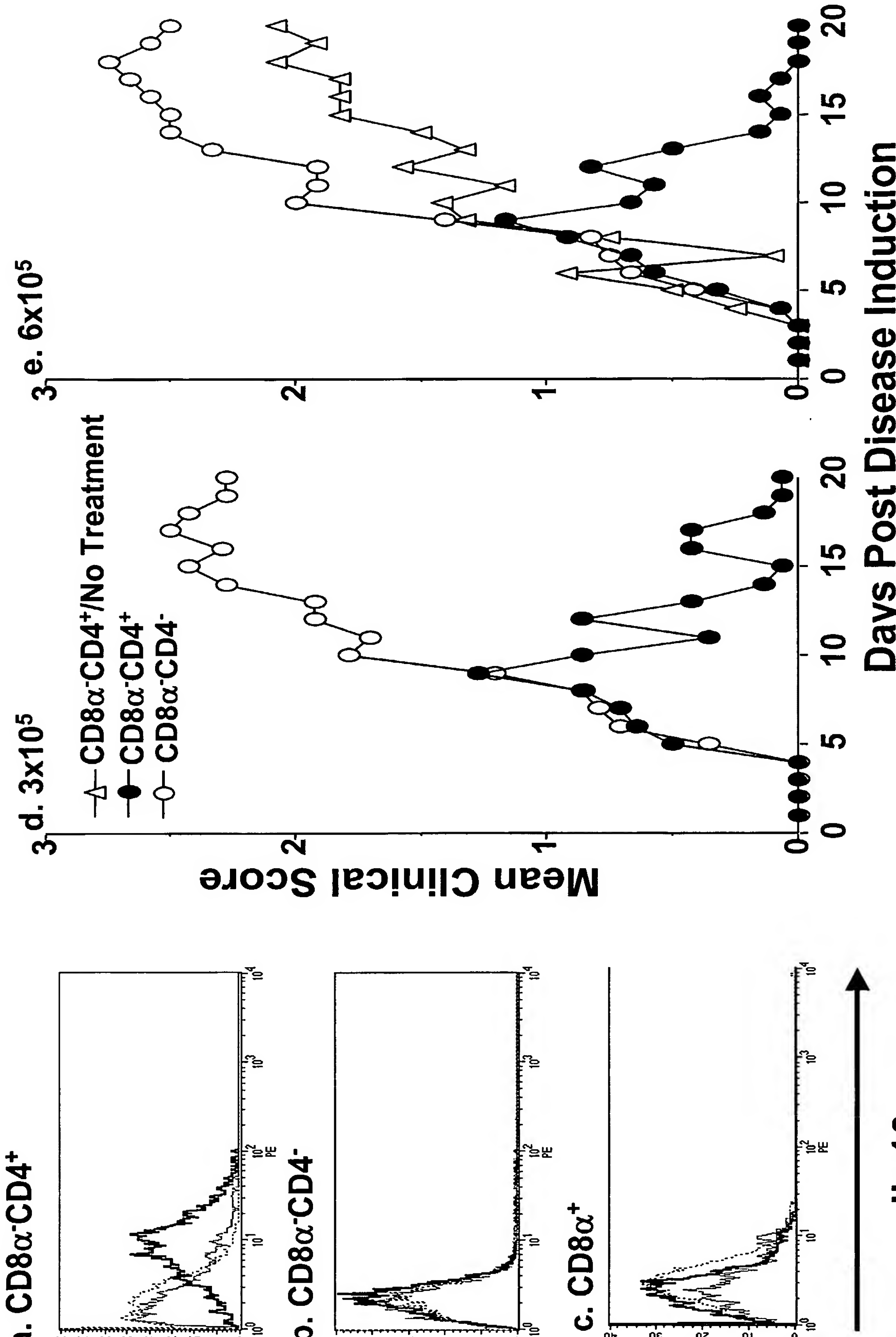

Mean Clinical Score
0
1
2
0
10
20
30
40
50
Days Post Disease Induction
CD8 α+
CD8 α-

Fig 9.


a. CD8α-CD4+
b. CD8α-CD4-
c. CD8α+
Counts
PE
IL-10
d. 3x10^5
e. 6x10^5
CD8α-CD4+/No Treatment
CD8α-CD4+
CD8α-CD4-
Mean Clinical Score
Days Post Disease Induction